A Taxonomic and Ecological Study of Periphytic Cyanobacteria in Kaituna River and Its Tributaries, Banks Peninsula, New Zealand

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For Eiman and Dania

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Abstract

Most of the detailed studies on periphyton in New Zealand rivers and streams have focused on diatoms. Despite the recent rise of interest in potentially toxic cyanobacterial mats, knowledge of the diversity and ecology of these and other macroscopic growth forms is incomplete.

A taxonomic survey was made on periphytic cyanobacteria at 100 locations along Kaituna River and a 1st to 3rd order tributary stream. Samples were taken from runs, riffles and pools in shaded and unshaded locations and from varied substrata from January to December 2011. Descriptions were made of all macroscopic growths. Fifty-six morphospecies were identified of which 29 are new records for New Zealand. Crust components were the most diverse with 23 morphospecies followed by mats (16), gelatinous colonies (5) and epiphytes (7). Five appeared only after growth in enrichment cultures.

Twelve morphospecies were isolated into cultures for use in polyphasic assessment. In 16S rDNA phylogenies, *Placoma regulare* and *Heteroleibleinia fontana* did not cluster with other members of their traditional families. *Nostoc* sp. 2 was positioned distant from other *Nostoc* strains. Comparison of 16S – 23S rRNA internal transcribed spacer compositions for seven mat-forming oscillatorialean morphospecies confirmed their recognition as distinct morphospecies. Amplified fragment length polymorphisms were used to investigate genetic diversity of *Nostoc verrucosum* in relation to dispersal. This indicated that local dispersal is dominant while cross-catchment dispersal is probably infrequent.

Light intensity, substratum type and water conductivity were significant factors influencing spatial patterns of distribution. Higher diversity of crusts, mats and gelatinous colonies was recorded in unshaded locations. Mats and gelatinous colonies were most diverse in Kaituna River and crusts in second to third order streams. Morphospecies in water with high conductivity were restricted to those locations. Spates had a major effect on temporal distribution. An increase in frequency and intensity of spates in spring and winter resulted in greater reduction in cover. Smaller spates caused partial removal followed by rapid regrowth within a week. Major spates caused complete removal of visible cover with re-colonization occurring within three to four weeks.

This study has provided a first detailed account of cyanobacterial diversity and ecology in a New Zealand catchment. It provides a basis for long-term monitoring at this site of the effects of changes in climate and in human activities in the catchment.

Chapter 1

General introduction

1.1 The context of this study

The distribution and diversity of cyanobacteria have not been intensively studied in New Zealand flowing waters (Broady and Merican, 2012). They have been reported as the least common component of stream periphyton after diatoms and green algae (Biggs, 2000b). In many studies, just prominent taxa have been identified to only generic level (Biggs *et al.*, 1998; Biggs and Kilroy, 2000; Biggs and Smith, 2002; Jaarsma *et al.*, 1998). More recently, benthic mat-forming cyanobacteria capable of toxin production have been widely studied (Hamill, 2001; Heath *et al.*, 2010; Wood *et al.*, 2007; 2008; 2010; 2012; Smith, 2010; 2012; Smith *et al.*, 2011). Other important growth forms have received scant attention. These include crusts, tufts, gelatinous colonies and epiphytic communities.

Under–sampling and taxonomic uncertainties have resulted in lack of knowledge of the diversity of periphytic cyanobacteria and environmental factors governing their distribution in New Zealand. Previous floristic and ecological studies of periphyton have not specifically focused on cyanobacteria. This has hampered discovery of new taxa that could produce toxins or useful bioactive compounds (Broady and Merican, 2012) and has restricted their use as indicators of water quality. A full knowledge of New Zealand's biodiversity requires a complete inventory of these widespread microorganisms.

The majority of taxa recorded in New Zealand are well-known and cosmopolitan morphospecies while poorly known and possibly endemic species have likely been overlooked (Broady and Merican, 2012). The description of a new species of a colony-forming cyanobacterium, *Placoma regulare* Broady & Ingerfeld, that is widespread in South Island streams (Broady and Ingerfeld, 1991), suggests that others await discovery.

Environmental factors affecting growth and distribution of cyanobacterial periphyton in particular are largely unknown despite numerous detailed studies on New Zealand stream periphyton in general (Biggs, 1990; Biggs and Price, 1987; Biggs *et al.*, 1998; Biggs and Smith, 2002; Jowett and Biggs, 1997; Suren *et al.*, 2003). The ability of cyanobacteria to detect and respond to a variable environment is well-known, e.g. to a wide range in light intensity and spectral quality (Montgomery, 2007; Mullineaux, 2001), periods of desiccations (Garcia-Pichel and Pringault, 2001; Hill *et al.*, 1997), nutrient limitations (Mateo *et al.*, 2006; Whitton, 2008), and grazing pressure (Jang *et al.*, 2007; Yang *et al.*, 2008). Distribution and diversity of cyanobacteria are potentially reliable indicators of water quality in streams and rivers impacted by anthropogenic environmental change

(Perona *et al.*, 1998; Douterelo *et al.*, 2004; Perona and Mateo, 2006; Loza *et al.*, 2013a). Additionally, morphological changes observed in some morphospecies are also an effect of environmental change, especially nutrient availability (Whitton, 2008). An increase in knowledge of their responses would enhance the use of cyanobacterial assemblages in monitoring the health of New Zealand flowing water ecosystems.

1.2 Project aims and an overview of the structure of this thesis

'What grows where?' is the primary question addressed by this research. This study has intensively examined the diversity and distribution patterns of periphytic cyanobacteria in first to fourth order flowing waters of a single catchment on Banks Peninsula, Canterbury, New Zealand. Descriptions were compiled for the diversity of macroscopic growths of cyanobacteria in different microhabitats throughout the study site. Over one year, an assessment was made of the effects of environmental variables on broad and small scale distribution patterns.

Chapter 2 describes the study site and features of the catchment and climate. The results are presented in Chapters 3 - 5, each of which provides an introduction and discussion.

Chapter 3 describes the diversity of morphospecies of cyanobacteria in the study site. The results presented here respond to the '*What*' question by addressing morphospecies diversity using descriptions of macroscopic growths and light microscopy of field-collected specimens and, in some cases, isolates studied in culture. Descriptions include photomicrographs and line illustrations.

Chapter 4 reports on the molecular phylogenetics of selected cyanobacteria. This responds to the need for polyphasic study in order to fully characterise and reliably identify many morphospecies. 16S rDNA phylogenies and ITS compositions have been obtained for all isolated strains and results compared to identifications made using the traditional approach (Chapter 3). The influence of dispersal on genetic diversity has been investigated using the technique of AFLP (amplified fragment length polymorphism).

Chapter 5 describes spatial and temporal patterns in the distribution of macroscopic growths of cyanobacteria. The results help answer the '*Where*' question by investigating distribution in relation to different environmental variables. Distinct patterns in morphospecies distribution have been observed in space and time and discussion focuses on environmental variables that might be of significance.

This study has increased the knowledge of cyanobacterial periphyton in New Zealand. Chapter 6 briefly summarises and discusses the main results and presents suggestions for further work.

Chapter 2

Description of the study site

2.1 Features of Kaituna Valley

Kaituna Valley is located in the south-west of Banks Peninsula. The Peninsula extends from the eastern edge of the Canterbury Plains into the Pacific Ocean (Wilson, 2009) (Fig. 2.1) and is approximately 50 km long by 30 km wide on, (Wilson, 2009) with an area of about 100,000 ha (Ogilvie, 1990).

Figure 2.1: Locality map of study site. **a**, New Zealand and location of Banks Peninsula boxed in red. **b**, location of Kaituna Valley outlined in orange and the study site shown in blue. Scale bars: 700 km for a; 5 km for b. (Source: GoogleMaps)

Kaituna Valley contains a major catchment that drains into Te Waihora / Lake Ellesmere to the south (Namjou, 1988). It extends more than 11 km from Lake Ellesmere to Mt Herbert in the north. The valley floor is wider at the entrance becoming slowly and steadily higher and narrower towards the two highest points on the Peninsula, Mt. Bradley (855 m) and Mt. Herbert (920 m) (Miskell, 2007). Much of this narrow valley is surrounded by hills of more than 500 m altitude. Many small streams drain these slopes and feed Kaituna River (Miskell, 2007).

2.1.1 Geology

Banks Peninsula comprises mainly of lava flows and other volcanic products of the deeply eroded remnants of two large and overlapping Miocene volcanoes – Lyttelton in the north-west and Akaroa

in the south-east – as well as several minor volcanic centres (Miskell, 2007). Eruption of the Lyttleton Volcanic Group began about 12 million years ago (Sewell *et al.*, 1993).

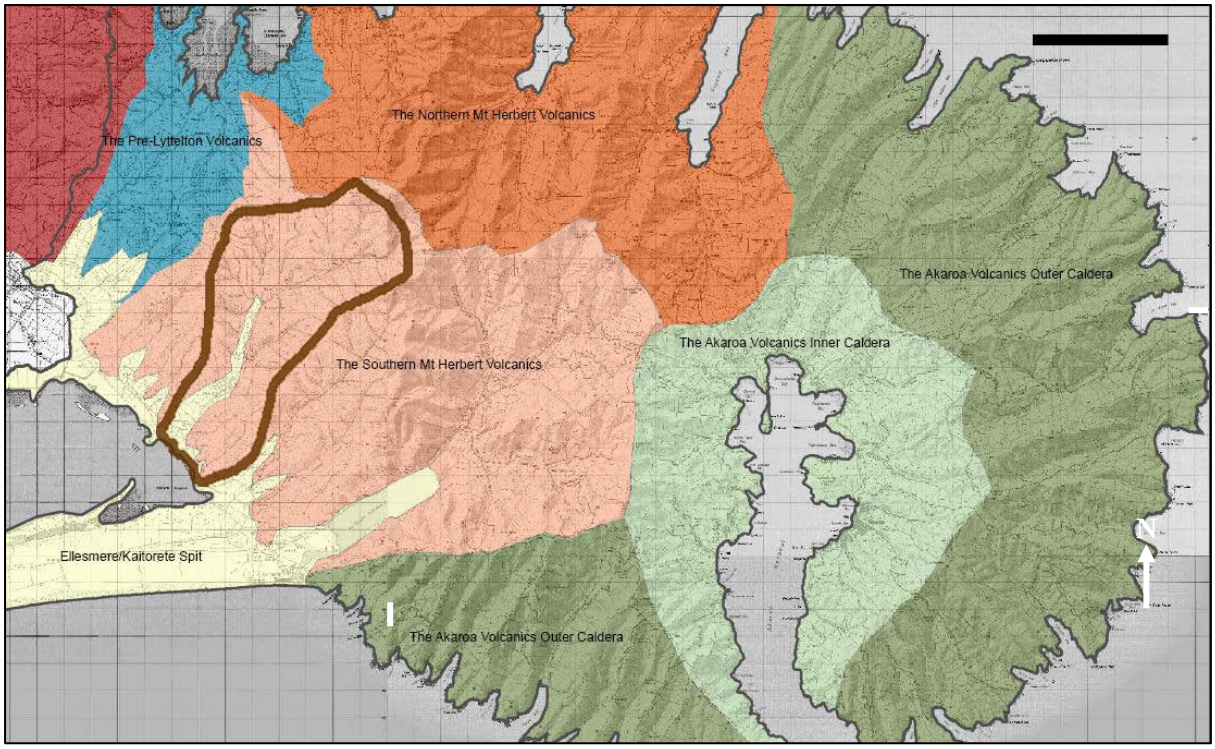

Figure 2.2: Geology of Kaituna Valley (outlined in brown) showing Mount Herbert Volcanic Group basaltic rocks being the most significant rock form. Scale bar: 5 km. (Source: Miskell, 2007).

Volcanic activity shifted to the south-east about 9.7 to 8.0 million years ago with the eruption of the Mount Herbert Volcanic Group (Sewell *et al.*, 1993). At about the same time, volcanism was also occurring in the Akaroa area. The final volcanic episode on the Peninsula occurred 7.0 to 5.8 million years ago resulting in the Diamond Harbour Volcanic group (Sewell *et al.*, 1993).

The Peninsula forms the largest accumulation of Coenozoic volcanic rocks in the South Island. These mostly comprise of basalts, andesites, trachytes and rhyolite (Wilson, 2009). The dominant rocks in the Kaituna River catchment comprise the Mt Herbert Volcanic Group. This includes basaltic lava flows and plugs, with minor interbedded volcaniclastic conglomerate, sandstone, siltstone, carbonaceous mudstone, and tuff (Sewell, 1988). These rocks form a significant part of central Banks Peninsula (Sewell, 1988) and a large proportion of the high ground, mid-slope and broad ridges of Kaituna Valley (Fig. 2.2). The soils on the Peninsula are derived from igneous bedrock and loess, with fertile alluvial soils on valley floors (Wilson, 2009).

2.1.2 Climate

The climate of Banks Peninsula is quite different from that of the adjacent Canterbury Plains. Rainfall increases considerably with altitude and there is a marked winter maximum (Wilson, 2009). Average annual rainfall for Kaituna Valley ranges from 600 mm close to Te Waihora to 2000 mm in

the upper catchment (R. Lough, 2012 pers. comm.). The main rain-bearing winds are from the south-west and south-east (Wilson, 2009). The predominantly easterly winds are variably rain-bearing (Wilson, 2009). South and south-west winds in winter bring more persistent rain and winter conditions can stretch into spring (Namjou, 1988).

Snow can lie for up to several days at altitudes of 500 - 600 m (Wilson, 2009). During nights in winter, ground frost can be extensive with the exception of some areas near the coast (Wilson, 2009). Rainfall is lowest in summer (Namjou, 1988). Dry, warm, north-west winds aggravate drought conditions (Wilson, 2009). During these periods any rainfall is soon evaporated (Namjou, 1988).

2.1.3 Vegetation

Fossil records indicate that podocarp, hardwood and southern beech trees (*Nothofagus* species) and ferns were significant elements of Banks Peninsula forests during the Miocene (Wilson, 2009). Beeches were mostly confined to the north-eastern parts of the Peninsula while mixed podocarp-hardwood forests dominated most of the remainder (Wilson, 2009).

Vegetation types occurring on the Peninsula today include tiny remnants of old-growth forest, about 9000 ha of second-growth hardwood forest dominated by kānuka and more than 6000 ha of mixed canopy consists of fuchsia, fivefinger, lemonwood, kōwhai and kaikōmako (Wilson, 2009). Vegetation in Kaituna Valley today consists of a heterogeneous matrix of farmland with significant areas of native bush and tussock grassland higher up the valley (Fig. 2.3). Several forest remnants exist including Mount Herbert Scenic Reserve, Kaituna Spur Scenic Reserve and Parkinson Bush which contain examples of the original podocarp-hardwood forest. Extensive areas of agricultural and horticultural land dominate the landscape (Fig. 2.3).

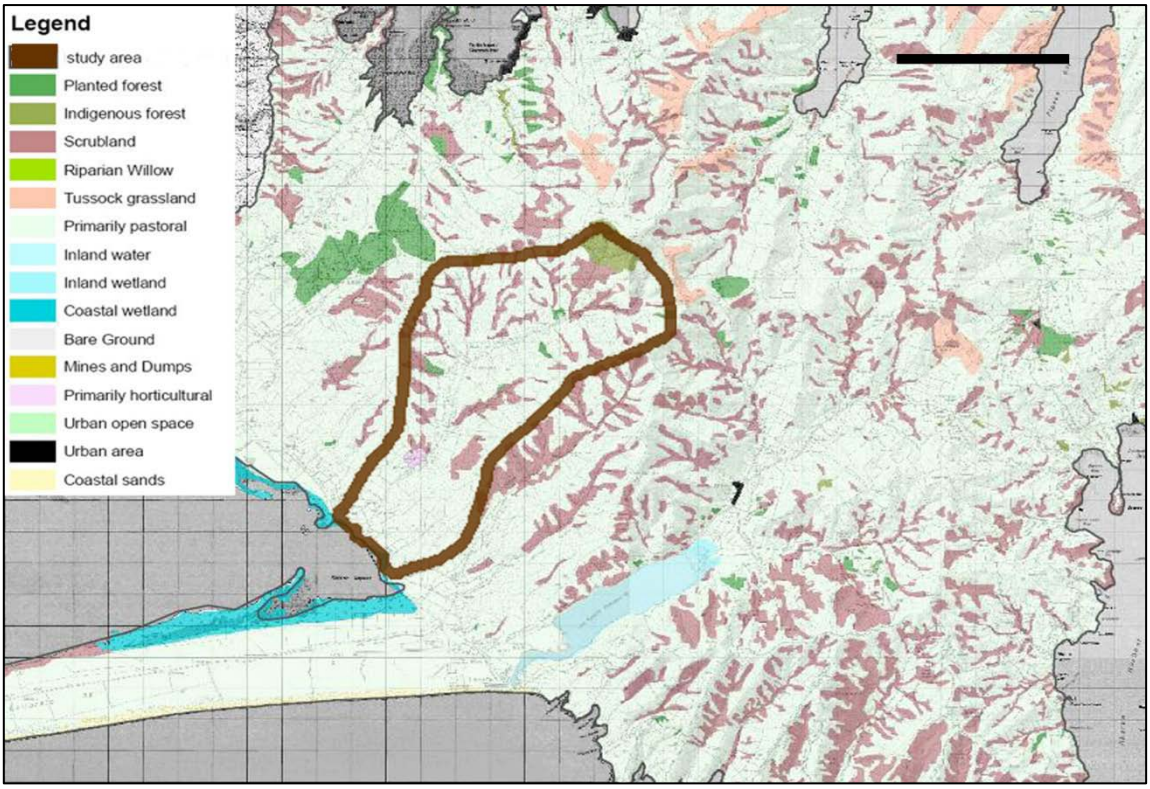

Figure 2.3: Present patterns of vegetation in Kaituna Valley (outlined in brown). Scale bar: 5 km. (Source: Miskell, 2007).

2.1.4 Human history and land use

The first settlers on the Peninsula were Polynesians (Ogilvie, 1992). They were hunter-gatherers and depended on the natural resources of the land for food supply. Their hunting activities caused extinction of several bird species and greatly reduced seal populations (Wilson, 2009). Approximately one third of the forest cover was cleared to make way for cultivation and building of pā (fortified settlements) (Wilson, 2009).

By the early 1830's, Europeans were living on the Peninsula with most of them being whalers, flax traders and deserters from ships (Ogilvie, 1992). Whaling stations were established on-shore, mostly along the south coast, by colonial whalers and whaling became Banks Peninsula's first major industry (Ogilvie, 1992). Whales and seals were driven to the verge of extinction by intensive hunting (Wilson, 2009).

Permanent European settlement began in 1840's with the arrival of French and German settlers in Akaroa (Wilson, 2009). The forest was exploited and large areas were destroyed by logging, clearance for grazing, and accidental and deliberate fires (Ogilvie, 1990). Most forest had been destroyed by 1900 (Petrie, 1963).

Cocksfoot grass seed production was a major industry on the Peninsula, during the 1840's (Ogilvie, 1992). Seeds were sold to 'bush burn' areas in the North Island and some were exported to Australia (Wilson, 2009). It continued to be a major industry into the early 1900's but the market declined with the end of bush clearance by burning in the North Island (Wilson, 2009).

Dairying was the mainstay of the Banks Peninsula economy from the late 1850's but since the 1950's meat and wool production have taken over as the main production industry (Wilson, 2009) (Fig. 2.4). Market gardening, fruit orchards and deer farming are also common on the Peninsula today (Fig. 2.4).

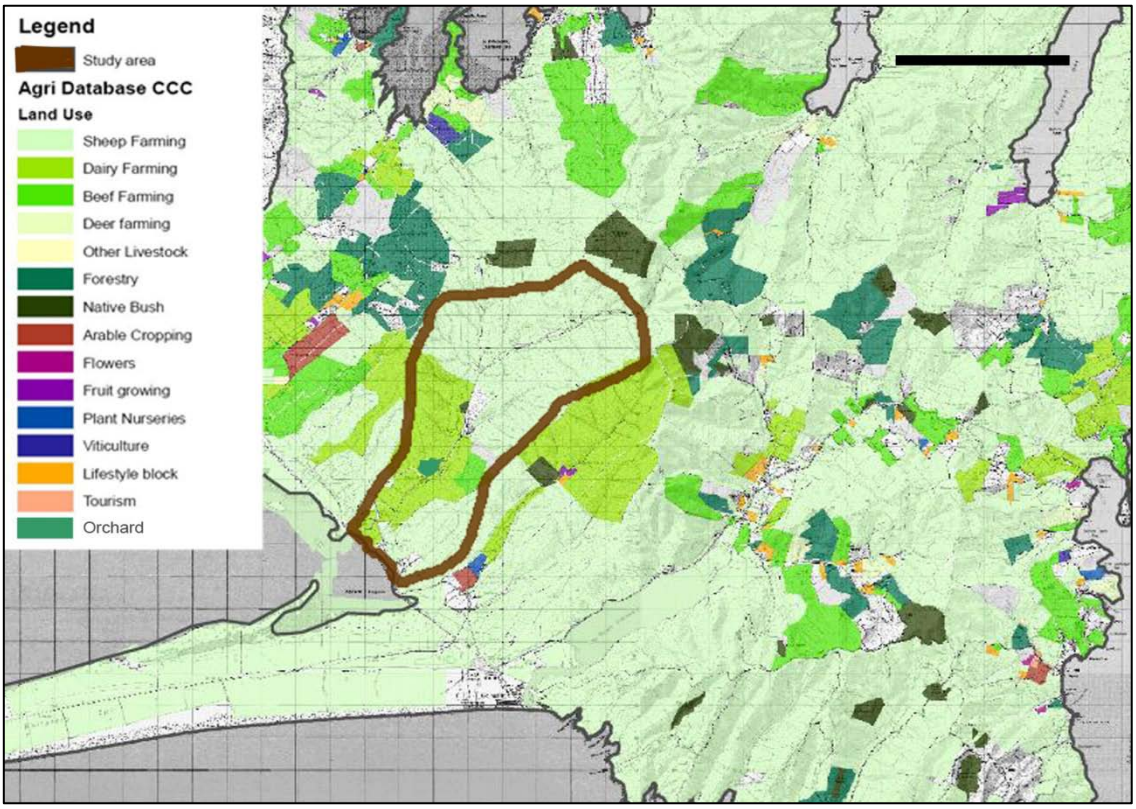

Figure 2.4: Meat and wool production and dairying are the main land use activities within Kaituna Valley (outlined in brown). Scale bar: 5 km. (Source: Miskell, 2007).

2.2 The Study Site

2.2.1 Topographical and physical characteristics

The study site included a stream in the valley-side catchment below Pack Horse Hut together with a reach of Kaituna River downstream from where this tributary joins the main river in the valley bottom. The site was divided according to different stream orders. Sampling locations were numbered from 1-100 with 1 being at the lower end of the site in Kaituna River and 100 at the highest altitude location. Sample location numbers along each stream order are indicated in Fig. 2.5a. Within each order, runs, riffles and pools were identified on the basis of water flow (Harding *et al*., 2009). The first and second order streams had a steep cascading flow with the gradient reducing through the

third order stream before changing into a much lesser gradient after confluence with the fourth order Kaituna River (Fig. 2.5b).

At approximately 420 m above sea level (a.s.l.), the first order stream flowed through Parkinson's Bush, a deeply shaded, fenced native forest remnant. The major part of this stream dried up completely during summer with only scattered small pools available at its lower end (Fig. 2.6a).

From the lower end of Parkinson's Bush at about 360 m a.s.l. and then down through the entire study site, water flow was present throughout the year (Fig. 2.6b). The substrata consisted predominantly of moss-covered bedrock and large boulders although some isolated areas with cobbles and silt were noted. The first order stream below the reserve remained heavily shaded and flowed through a steep bush-covered valley.

After a confluence at approximately 300 m a.s.l, the second order stream remained heavily shaded as it continued to flow through the steep bush-covered valley (Fig. 2.6c). The stream channel gradually increased in width and volume. The substrata remained predominantly moss-covered bedrocks and boulders with cobbles, gravel and silt becoming more common downstream. Bedrock included high, near vertical faces at higher elevation but slopes decreased downstream. At approximately 180 m a.s.l., the stream crossed a recreational track to Pack Horse Hut. The banks were completely exposed along the approximately 12 m stream length (Fig. 2.6d). Substrata were predominantly cobbles, pebbles and boulders. From approximately 240 m a.s.l. to this point onwards, the stream was accessible to livestock.

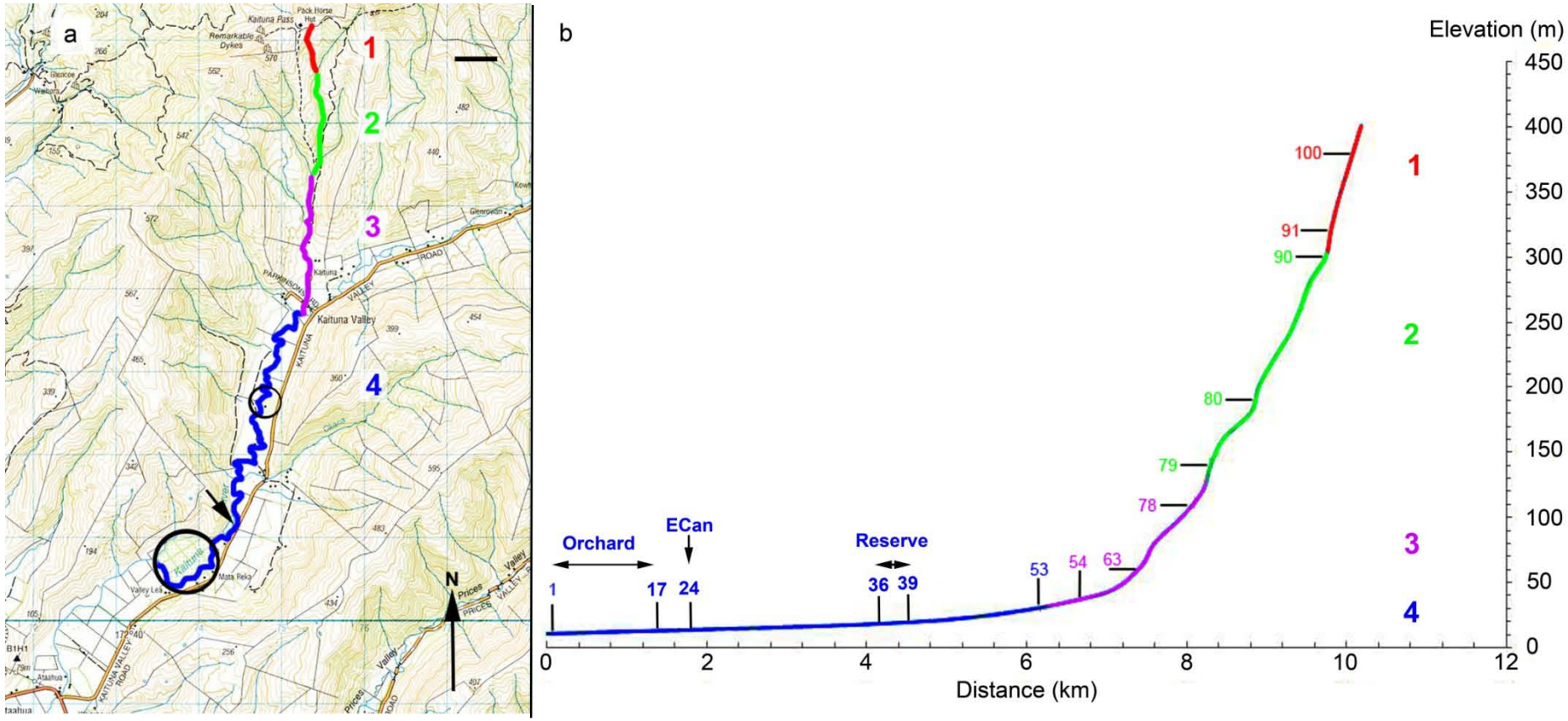

Figure 2.5: The study site. **a,** Increasing stream order (1-4) shown as different colours; black arrow indicates the Environment Canterbury (ECan) monitoring site located on the fourth order, valley bottom Kaituna River; Kaituna Valley Scenic Reserve and the orchard are circled in black; **b,** profile of the study site indicating stream orders by colour as in (a) and the positions of selected numbered sampling locations. Scale bar in (a) is 500 m.

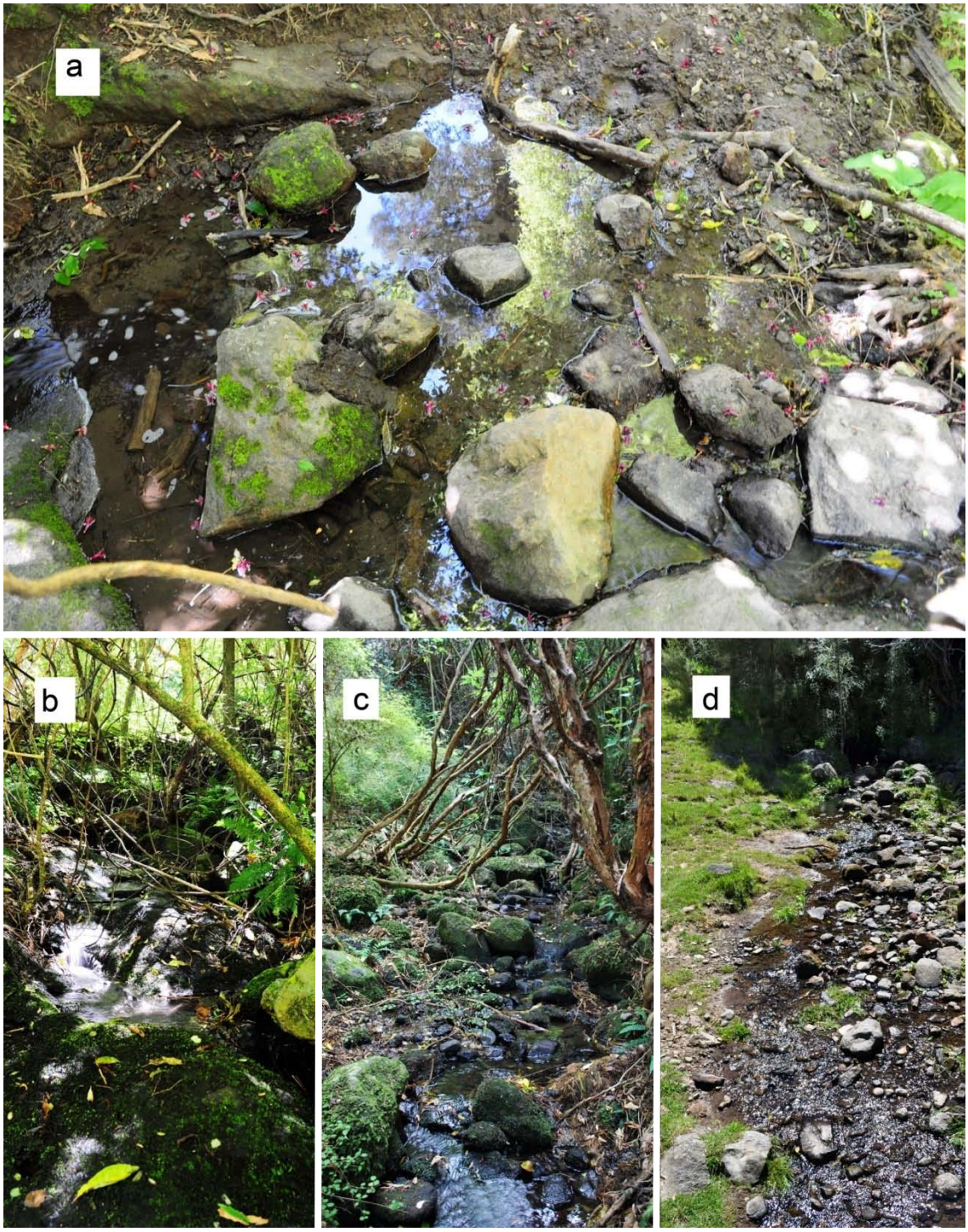

Figure 2.6: a, b, the first order stream. **a,** small pool of water flowing through Parkinson's Bush. **b,** more stable flow in the lower part of the deeply shaded bush. **c, d,** the second order stream. **a,** stream flowing through shaded bush-covered valley. **d,** unshaded stretch crosses a track.

The third order stream commenced at an elevation of approximately 120 m a.s.l. Stream width and depth continued to increase gradually. Riparian vegetation was still dense in most upper stretches (Fig. 2.7a) but decreased gradually downstream. The stretch further downstream was mostly unshaded (Fig. 2.7b, c) apart from short parts where it was shaded by high loess banks. Substrata were predominantly cobbles, pebbles, gravel and silt with boulders noted in some areas. Moss-covered rocks were more common in the upper shaded stretches. The catchment adjacent to this third order stream included a mix of extensive sheep and cattle pasture (Fig. 2.7c) with several woodlots of pine, macrocarpa and eucalyptus trees. There was little fertiliser or lime application to land and pesticide use was primarily for small areas of gorse (R. Lough, 2012, pers. comm.). The stream flowed approximately 2 km before confluence with Kaituna River in the valley bottom.

The fourth order part of the study site, in Kaituna River, started at an elevation of approximately 35 m a.s.l. Riparian vegetation was greatly reduced (Fig. 2.7d) apart from the stretch in Kaituna Scenic Reserve. The river was considerably wider than the tributary stream with channels up to approx. 7 m wide. Relatively smooth runs were common between rapid riffles. Pools were larger and deeper with some exceeding one meter depth. Substrata were predominantly cobbles, pebbles and silt (Fig. 2.7d) although boulders were present in some fast flowing riffles.

Stream bed morphology changed completely to silt in lower stretches and supported extensive growths of aquatic angiosperms (Fig. 2.7e). The channels became slower flowing moving downstream and were silted in the lower stretch where the river flowed out through orchards. Bank erosion was evident at a number of locations possibly due to lack of riparian vegetation and livestock access.

The Kaituna River then flows through areas of intensive livestock farming, farmland with supplementary crops and hay making, and orchards (R. Lough, 2012, pers. comm.). Lime, fertilisers and agricultural pesticides were regularly used on these areas

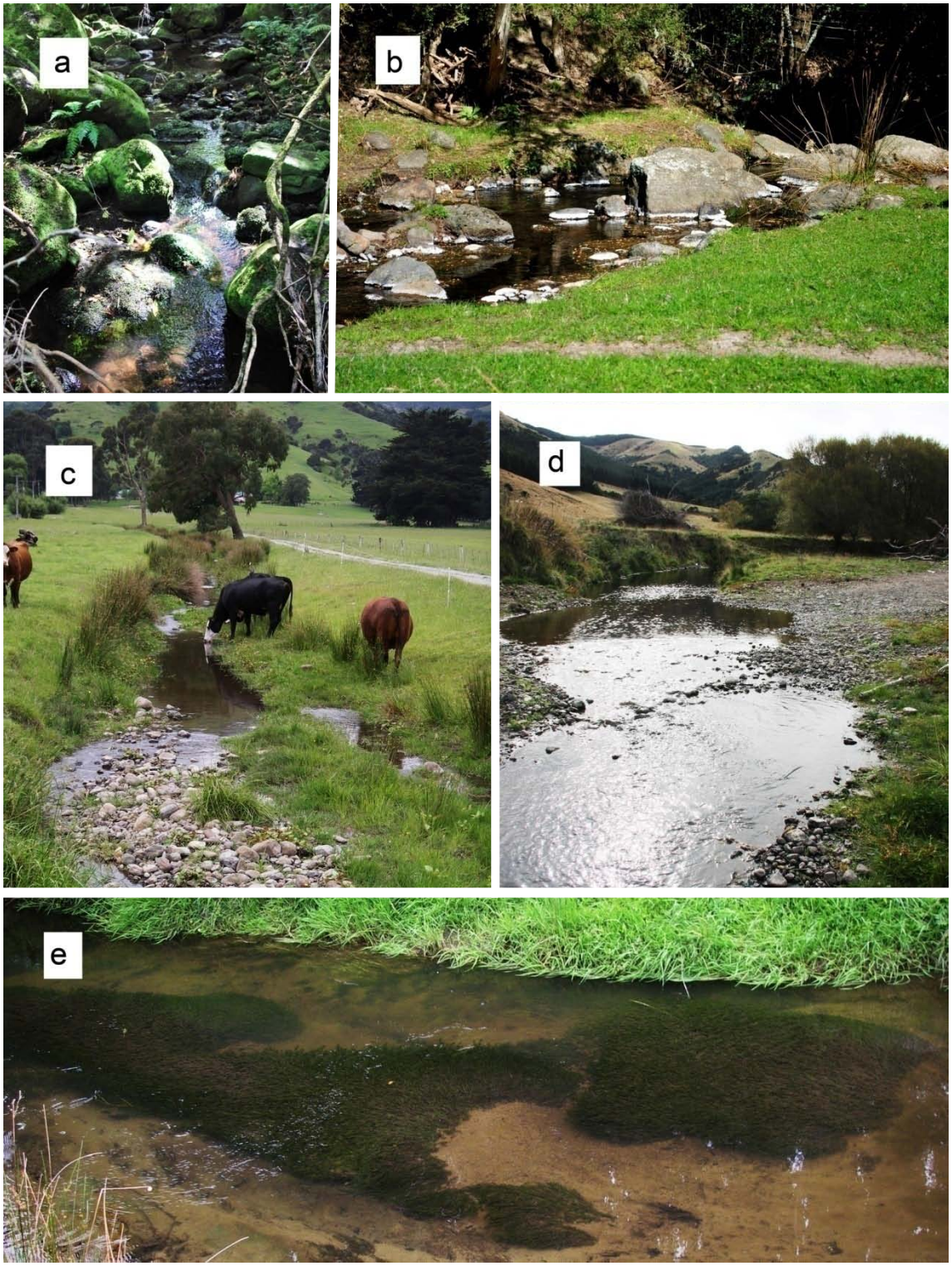

Figure 2.7: a-c, the third order stream. **a,** shaded upper stretches flowing through bush-covered valley. **b, c,** lower stretch flowing through unshaded and greatly modified catchment. **d, e,** the fourth order valley bottom river. **d,** unshaded Kaituna River with bed morphology of cobbles. **e**, further downstream with river bed comprised of fine-grained sediment supporting extensive growths of aquatic macrophytes.

2.2.2 Water Quality

Water temperature, dissolved oxygen and nutrient concentration data were available for Kaituna River from monthly water quality monitoring from 1992 – 2010 by Environment Canterbury at a location on the fourth order stream (Fig. 2.5). A summary of this data is presented in Table 2.1.

Table 2.1: Median (range in brackets) water quality data* for Kaituna River at the Environment Canterbury recording station on Kaituna River (fourth order flow).

Duration of records (years)	Temperature (˚C)	Dissolved oxygen (%)	Nitrate + nitrite nitrogen (g/m^3)	Dissolved reactive phosphorus (g/m^3)
17 (1992 - 2010)	10.95 (8.16 - 13.13)	98.30 (81.89-118.05)	0.14 (0.03-0.65)	0.02 (0.010-0.040)
Natural Resources Regional Plan Standards	<20	>90	<0.09	<0.025

*Data is from (Associates, 2011)

Water temperature was usually highest in January/February (15.3-21.9°C) and lowest in June to August (4.4-8.9°C). Median water temperatures based on spot readings were low and seldom exceeded the temperature standard (20°C) for protection of aquatic life in Canterbury rivers (Associates, 2011). However, continuous temperature data for January to February 2009 showed the maximum daily temperature frequently exceeded the standard daily limit of 20°C (Fig. 2.8). This is thought to reflect typical summer temperatures at this location as the summer of 2008/2009 was not unusually hot (Associates, 2011). Kaituna River is considered to be sensitive to the effect of reduced flow in summer causing an increase in water temperature but this effect is less likely in winter (Associates, 2011).

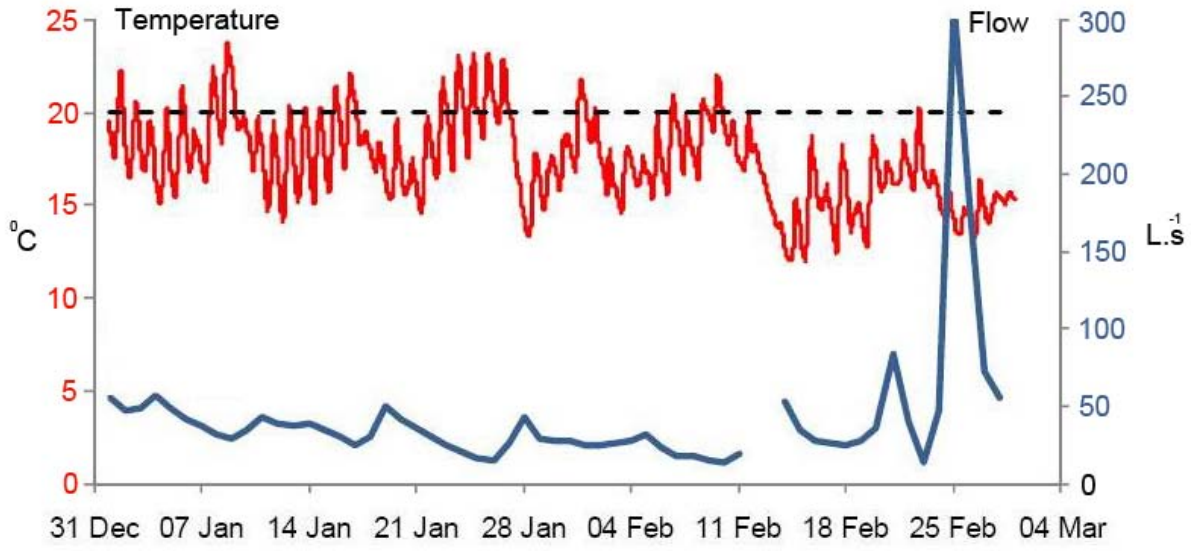

Figure 2.8: Temperature and mean daily flow data for Kaituna River in summer 2009 (15 minutes interval). A spike in flow occurred after heavy rain in late February. The dashed line indicates the 20°C temperature objective in the National Resources Regional Plan (adapted from Associates, (2011)).

Dissolved oxygen (DO) was maximal in September and November and lowest levels occurred in February to April (Associates, 2011). Median DO concentration exceeded the Natural Resources Regional Plan (NRRP) objective of 70% and 90% based on spot measurements (Table 2.1).

Median concentrations of nitrate + nitrite nitrogen (NNN) exceeded the NRRP standards (Table 2.1) and the periphyton guidelines values of Biggs (2000b) (Fig. 2.9). Enriched NNN concentrations in Kaituna River could reflect the influence of nitrate-enriched run off from the surrounding catchment dominated by the invasive weed, gorse (*Ulex europaeus*). This fixes nitrogen and produces large amounts of litter which releases nitrogen during decomposition (Guna *et al.*, 2012).

Overall median concentration of dissolved reactive phosphorus (DRP) was above the NRRP standards (0.025 g/m^{-3}) (Table 2.9). Concentrations of DRP plotted against the periphyton guideline values of Biggs (2000b) showed values exceeding the guidelines indicating greater likelihood of nuisance proliferations to occur in the river (Associates, 2011). Elevated nutrient concentrations reflect a pattern of intensive agricultural activity in the lowland catchment (Hayward *et al.*, 2009).

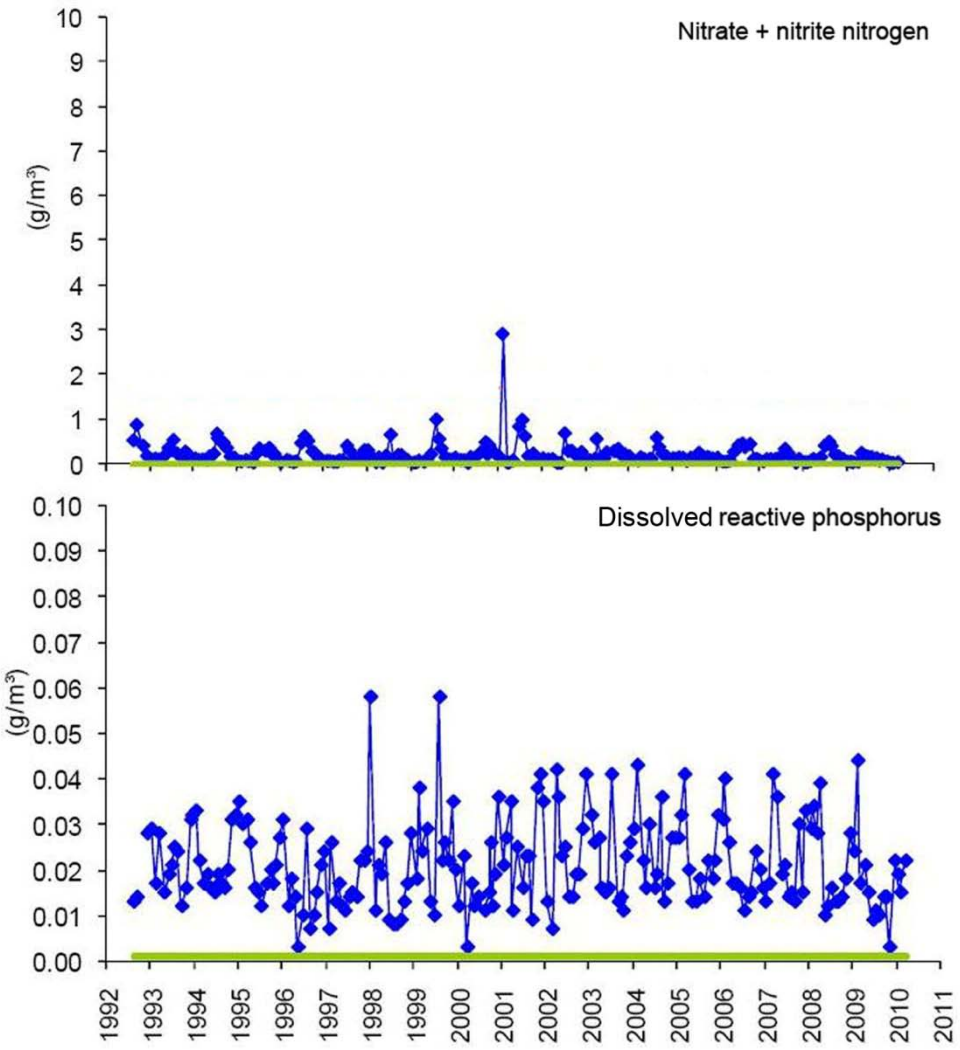

Figure 2.9: High concentrations of Nitrate + nitrite nitrogen and dissolved reactive phosphate in Kaituna River exceeding the nutrient guidelines values for the prevention of nuisance periphyton growth (green line) during the period of 1992-2010 (Associates, 2011).

2.2.3. Flow

Kaituna River has a flow regime that is fairly typical of a moderately flood-disturbed hill-fed river (Associates, 2011). Flow rate responds rapidly to heavy rainfall events that occur more frequently in winter (Fig. 2.10). Due to catchment elevation and volcanic geomorphology, the river experiences high flow events (e.g. Fig. 2.8) that rise and recede quickly (Taylor and Good, 2006). High flow events usually result in prolonged turbidity due to the volcanic loess soils (Taylor and Good, 2006).

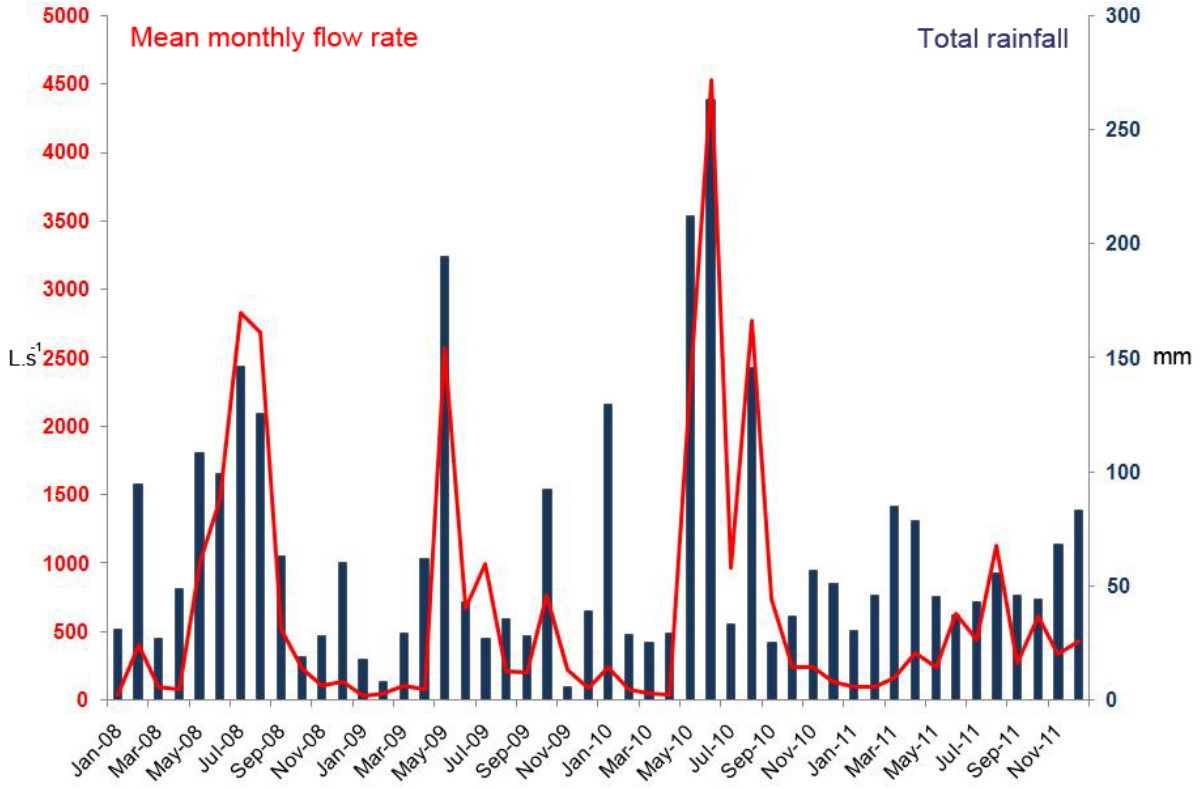

Figure 2.10: Monthly average daily flow (litres per second) and total rainfall (millimetres) in Kaituna River from 2008-2011 showing maximum flows typically in winter. The winter of 2011 was unusually dry. (Source: data from (Meredith), Environment Canterbury, Christchurch).

Chapter 3

The diversity of morphospecies of Cyanobacteria

3.1 Introduction

3.1.1 Prior research on cyanobacteria in New Zealand freshwaters

New Zealand freshwater cyanobacteria were first recorded by Nordstedt (1888) and Lemmermann (1899). Nordstedt (1888) identified approximately 90 taxa of cyanobacteria from algal collections made throughout New Zealand whilst Lemmermann (1899) listed 18 taxa from the South Island and Cook Strait islands. Since then, many studies have added to the total number of known cyanobacteria. The increase in knowledge is apparent from the series of checklists by Chapman *et al.* (1957), Chapman (1975), Skuja (1976), Cassie (1984) and Broady and Merican (2012).

In 1987, an international forum on Cyanobacteria held at the University of Waikato resulted in a special issue of the New Zealand Journal of Marine and Freshwater Research (Cooper, 1994). This included records of 104 taxa of planktonic cyanobacteria from a major survey conducted in 77 lakes (Pridmore and Etheredge, 1987). Detailed descriptions and illustrations were then provided by Etheredge and Pridmore (1987). Most of the taxa recorded were cosmopolitan species of Nostocales and Chroococcales (Cooper, 1994). Although inadequate sampling was acknowledged (Pridmore and Etheredge, 1987), the outcome of the study greatly increased the knowledge of cyanobacteria diversity and distribution in New Zealand.

Periphytic cyanobacteria remained poorly known. Many studies have subsequently been conducted on stream and river periphyton (Biggs, 1990;1995;1996;2000a; Biggs and Price, 1987; Biggs *et al.*, 1998). Diatoms were the most studied group (Biggs, 1995; 2000a). Records of benthic cyanobacteria were sparse and comprised identifications to only the generic level (Biggs, 1990b; Biggs *et al.*, 1998; Biggs and Smith, 2002). Benthic mat-forming cyanobacteria capable of toxin production are by far the most widely studied (Hamill, 2001; Heath *et al*., 2010; Smith, 2010; 2012; Smith *et al.*, 2011; Wood *et al.*, 2007; 2008; 2010; 2012).

At present, 367 morphospecies from 23 families representing the four orders, Chroococcales, Oscillatoriales, Nostocales and Stigonematales are known from terrestrial and freshwater habitats (Broady and Merican, 2012). This includes at least 36 doubtful records and the total is estimated to be about 200 short of a full inventory (Broady and Merican, 2012). More than half of the morphospecies recorded have not been illustrated and many more than this lack voucher specimens in herbaria (Broady and Merican, 2012).

3.1.2 Approaches to cyanobacterial taxonomy

Traditionally, cyanobacteria were classified based on morphological features of field-collected specimens using light microscopy (Desikachary, 1959; Geitler, 1932). Specimens with similar phenotypic appearance were placed under the same species. This has resulted in the identification of over 2000 morphospecies in about 150 genera (Waterbury, 2006) Many morphospecies were separated by only a single character.

A different approach was suggested by Drouet (1968; 1978; 1981). He believed that most traditional morphospecies were environmentally induced variants of a limited number of genotypes. He greatly reduced the number of species and genera to 62 and 24 respectively (Castenholz, 2001). The application of this approach through the 1970s and 1980s in particular led to the loss of valuable ecological information (Whitton, 2008). Modern studies using strains isolated into culture combined with the application of molecular phylogenetics has shown Drouet's approach to be untenable.

Stanier *et al.* (1971) established the so-called "bacteriological approach" to the classification of cyanobacteria. This was based on morphological, physiological, biochemical and genetic characteristics of cultured, axenic and clonal strains. Although this provides additional information which is very useful for reliable identifications (Whitton, 2011), cultures represent only a very small proportion of the species that probably exist in nature. This approach is of limited use in floristic and ecological studies.

Discrepancy between the traditional and bacteriological approaches resulted in the proposed combination of morphological, genetic, ultrastructural and ecological information to adequately characterise and confidently identify different taxa (Komárek and Anagnostidis, 1989; 1999; 2005). This 'polyphasic approach', defines a morphospecies as a group of populations belonging to the same genotype, recognisable by a stable phenotype and occurring in ecologically similar localities (Komárek, 2011). The approach has been widely used in recent cyanobacterial studies (Boutte *et al.*, 2005; Ballot *et al.*, 2008; Berrendero *et al.*, 2008; Zapomělová *et al.*, 2009; 2010; Heath *et al.*, 2010; Dadheech *et al.*, 2012; Sciuto *et al.*, 2012; Loza *et al.*, 2013a; Martineau *et al.*, 2013).

3.1.3 Aims

The diversity of morphospecies has been investigated to achieve the following.

1. Thorough descriptions of morphospecies based on descriptions of visible growths in the field and on light microscopy of field-collected specimens.
2. Combination of morphological description of cultured isolates and field material wherever possible to aid identification.
3. Comparison of the diversity of the cyanobacterial flora at the study site with that recorded by other studies in similar environments.

3.2 Methods

3.2.1 Sampling procedure

A floristic survey was conducted from January to December 2011. An initial one off survey was conducted at the beginning of the study to identify all the different macroscopic growth forms along the whole system. All accessible locations along each stream order were included in the survey. A total of 100 sampling locations were identified for subsequent monthly survey (Chapter 5). In each stream order, runs, riffles and pools were examined and samples of visible growths of cyanobacteria were taken from different substrata. Substrata sampled were bedrocks, boulders, cobbles, pebbles, gravels and silt. Autoclave-sterilised spatulas were used to scrape crusts from rock surfaces. Gelatinous colonies, cyanobacterial mats, other macroalgae and other vegetation (angiosperms, bryophytes, *Vaucheria*, filamentous chlorophytes and rhodophytes) for epiphytic cyanobacteria were collected by hand. Each sample was stored with water from the collecting site in separate sterile polycarbonate screw-top containers (60 mL). These were kept chilled in an ice chest and transported back to the laboratory where they were refrigerated overnight before analysis.

3.2.2 Analysis of samples

3.2.2.1 Light microscopy of field material

Crust samples were vigorously shaken by hand and a small portion of the sample removed using a Pasteur pipette. Gelatinous colonies were broken up into small portions. Mats and tufts were teased apart using needles under a dissector microscope (Wild M13Z). Slide preparations for microscopy were of fresh material mounted in water. Cover glasses were sealed to the slide using nail polish in order to avoid evaporation during observation. Each slide was prepared as thin as possible to ensure good quality images.

Observations were made using an Olympus BX50 microscope at 100-2000x magnification. Illustrations were made with the aid of *camera lucida*. Each entire preparation was examined and all morphospecies of cyanobacteria present were recorded. New preparations of each sample were

examined until two consecutive slides contained no additional morphospecies. All relevant morphological features were recorded for each morphospecies. Size measurements were made on 50 replicate randomly chosen specimens for each morphospecies. These included: vegetative cell width and length, heterocyte and akinete width and length, and filament width. Descriptions, illustrations and photomicrographs were compiled for all morphospecies encountered.

3.2.2.2 Establishment of cultures

BG-11 and BG-11_0 (lacking chemically combined nitrogen) 1% agarised medium (Rippka *et al.*, 1979) with 100 µg/mL cycloheximide were prepared as follows. For every litre of final medium, 500 mL double strength medium and 250 mL 4% agar were autoclaved separately, cooled to approx. 45°C and then combined. A 250 mL filter- sterilised (pore size 0.25µm) cycloheximide solution (400 µg/mL) was added to the cooled but still molten medium. The medium was then poured into 50 mm diam. sterile plastic Petri plates and left to set in a laminar flow cabinet. Cycloheximide was used to help eliminate eukaryotic microbes present in inocula (Bolch and Blackburn, 1996).

Three replicate plates were inoculated with material from each sample. Samples containing scrapings from crusts and tufts, and small portions of gelatinous colonies were streaked over the agar surface using a flame-sterilised loop while small portions of mats were inoculated in the centre of plates. Plates were sealed with Parafilm® M sealing film to avoid evaporation. A small subsample of each sample was also inoculated into sterilized polycarbonate screw-top (60 mL) containers with 30 mL liquid BG-11 and BG-11_0.

Plate cultures inoculated with mat samples were examined after a week of incubation (18 ± 2 °C; 16:8 h light:dark at 25-100 µmol m^{-2} s^{-1} from cool white fluorescent lamps). Single trichomes radiating from the central inoculum were removed under a dissector microscope by cutting out the piece of agar (approx. 0.1 mm) upon which the trichome rested. This was transferred onto a fresh plate of the same medium and left to incubate for 2-3 weeks.

Streaked plates were observed for visible single colonies after 3 weeks incubation. Colonies were examined under the light microscope and those resembling morphospecies present in field material were isolated into unialgal cultures. Morphospecies that grew in plate cultures but were not initially identified in field material were also isolated.

After three weeks of incubation, mixed species liquid cultures were examined microscopically.

Light microscopy of all cultures was as described in section 3.2.2.1 for field material.

3.2.3. Literature used for identification of morphospecies

The taxonomic system by Komárek and Anagnostidis (1989; 1999; 2005) has been used. Reference was also made to floras of Geitler (1932), Desikachary (1959), McGregor (2007) and Whitton (2011). Identifications were taken to the lowest taxonomic level possible using all available information. Where uncertainty exists it is indicated by "cf." (Latin *confertim* = to compare with). The use of the orthograph 'heterocyte' in place of 'heterocyst', as recommended by the IAC (Mollenhauer *et al.*, 1994) is followed here.

3.3 Results

3.3.1 Overview of all recorded morphospecies and their forms of macroscopic growth

Fifty-six morphospecies, including 18 Chroococcales, 24 Oscillatoriales and 14 Nostocales were identified (Table 3.1). Of these, 51 morphospecies were described from field material. Only 16 of these were isolated into clonal cultures. The remaining five were observed only in cultures. Twenty-nine morphospecies are new records for New Zealand.

Table 3.1: List of periphytic cyanobacteria identified. Different macroscopic growth forms and presence or absence in both clonal and mixed cultures are indicated. Specimens not forming visible growths but present as microscopic associates at low abundance are indicated by + and new New Zealand records by *. G.col. = gelatinous colonies

Morphospecies	Field	Cultures		Macroscopic form			Epiphytes
		Clonal	Mixed	Crust	Mat	G.col	
CHROOCOCCALES							
Aphanothece clathrata			+				
*Chamaesiphon amethystinus**	+						+
C. cf. *britannicus**	+			+			
C. cf. *confervicolus var. confervicolus*	+						+
C. incrustans	+						+
*C. subglobosus**	+			+			
Chlorogloea cf. *microcystoides**	+			+			
*Cyanodermatium fluminense**	+			+			
C. cf. *gigas**	+			+			
Cyanodermatium sp.*	+						+
Hydrococcus cf. *rivularis*	+			+			
Merismopedia punctata			+				
Placoma regulare	+	+				+	
Pleurocapsa cf. *minor**	+			+			
Pleurocapsa sp.	+			+			
Radaisia sp.*	+			+			
Xenococcus sp.*	+						+
Xenotholos cf. *kerneri*	+	+	+				+
OSCILLATORIALES							
Geitlerinema amphibium	+	+			+		
*G. ionicum**	+	+	+		+		
*Heteroleibleinia fontana**	+	+	+	+			
H. cf. *kossinskajae**	+			+			

Table 3.1. Continued.

Morphospecies	Field	Cultures		Macroscopic form			Epiphytes
		Clonal	Mixed	Crust	Mat	G.col	
H. cf. *pusilla**	+			+			
H. cf. *versicolor**	+			+			
*Homoeothrix gracilis**	+			+			
*Homoeothrix juliana**	+			+			
H. cf. *varians**	+			+			
Leptolyngbya cf. *bijugata**	+	+	+		+		
O. cf. *simplicissima**	+	+			+		
Phormidiochaete sp.*	+			+			
Phormidium autumnale	+	+			+		
P. cf. *bekesiense**	+	+			+		
P. cf. *chalybeum*	+				+		
P. inundatum	+	+			+		
P. cf. *irriguum**	+	+			+		
P. cf. *subfuscum**	+	+			+		
P. uncinatum	+	+			+		
Pseudanabaena cf. *amphigranulata**			+				
P. cf. *galeata**			+				
NOSTOCALES							
Anabaena cf. *inaequalis*	+				+		
A. cf. *oscillarioides*	+	+				+	
Calothrix braunii	+			+			
C. cf. *epiphytica**	+						+
C. parietina	+			+			
Calothrix sp.			+				
Cylindrospermum cf. *muscicola**	+				+		
Dichothrix sp.	+			+			
Nostoc verrucosum	+					+	
Nostoc sp. 1*	+					+	
Nostoc sp.2*	+	+				+	
Rivularia sp. 1	+			+			
Rivularia sp. 2	+			+			
Trichormus cf. *variabilis*	+	+			+		

Sixteen taxa were identified from 12 distinct types of mat. Most mat-formers were Oscillatoriales. These comprised seven morphospecies of *Phormidium*, three of *Oscillatoria*, two of *Geitlerinema* and a single *Leptolyngbya* (Table 3.1). Three morphospecies (*O. curviceps*, *G. ionicum* and *L. bijugata*) co-occurred within mats dominated by *Phormidium* morphospecies. Three representatives of mat-forming Nostocales have been identified (*Anabaena* cf. *inaequalis*, *Cylindrospermum* cf. *muscicola* and *Trichormus* cf. *variabilis*).

Crust components were the most diverse. Twenty-three morphospecies from all three orders were recorded from three crust types. Oscillatoriales and Chroococcales dominated crusts, each with nine morphospecies (Table 3.1). Almost all morphospecies that dominated crusts were new records for New Zealand. Nostocales were represented by five morphospecies of *Calothrix* and *Rivularia.*

Five morphospecies formed each of the five distinct types of gelatinous colonies. Colonies of *Nostoc verrucosum* and *Nostoc* sp. 1 were most abundant. The remaining three morphospecies, *Placoma regulare*, *Anabaena oscillarioides* and *Nostoc* sp. 2, were infrequent.

Filamentous rhodophytes supported the most diverse community of epiphytic cyanobacteria (*Chamaesiphon amethystinus, C.* cf. *confervicolus var. confervicolus, Cyanodermatium* sp. and *Xenococcus* sp.) whilst the remaining three epiphytes were found on filamentous chlorophytes and gelatinous colonies. No epiphytic cyanobacteria were recorded from the other macroalgae collected.

3.3.2 Descriptions of morphospecies

Presented below are descriptions, *camera lucida* illustrations and photomicrographs of all morphospecies listed in Table 3.1. Genera are listed alphabetically within each family and morphospecies within each genus. Literature used for identification is given below the name of each morphospecies. The written descriptions and the figures emphasise diagnostic morphological characters. The distribution of each morphospecies within the study site is briefly outlined (Chapter 5 provides more details). Finally, some taxonomic notes are provided for each morphospecies.

Family **Chamaesiphonaceae**

Chamaesiphon amethystinus (Rostafinski) Lemmermann 1910
Komárek and Anagnostidis (1999): p379, fig. 490 (p378)

Description: Field specimens sparsely distributed and attached to sporophyte-stage of the filamentous rhodophyte *Batrachospermum* (Fig. 3.1a). Cells brownish, cylindrical, basally attached to the substratum (Fig. 3.1b), 3.0-6.0 µm wide, 4.0-18.0 µm long, broadly rounded at the apex, slightly widened and blunt towards the base (Fig. 3.1f), straight to very slightly bent. Cell content uniform. Exospores 1 or 2 in a row at the apex of the mother cell, spherical, 2.0-4.0 µm diam. Pseudovagina thin, firm, hyaline with distinct basal gelatinous pad (Figs. 3.1b, f).

Occurrence: Epiphytic in shaded second order stream.

Remarks: Specimens differ from *C. amethystinus* as described by Komárek and Anagnostidis (1999) in only the absence of granules. In older accounts, the presence or absence of granules was a diagnostic feature in some species of *Chamaesiphon* but this has been shown to be dependent on environmental conditions (Whitton, 2011).

Chamaesiphon amethystinus has previously been found growing epiphytically on *Batrachospermum* and filamentous cyanoprokaryotes in clear streams from temperate zones and tropical regions, usually in limestone areas (Komárek and Anagnostidis, 1999).

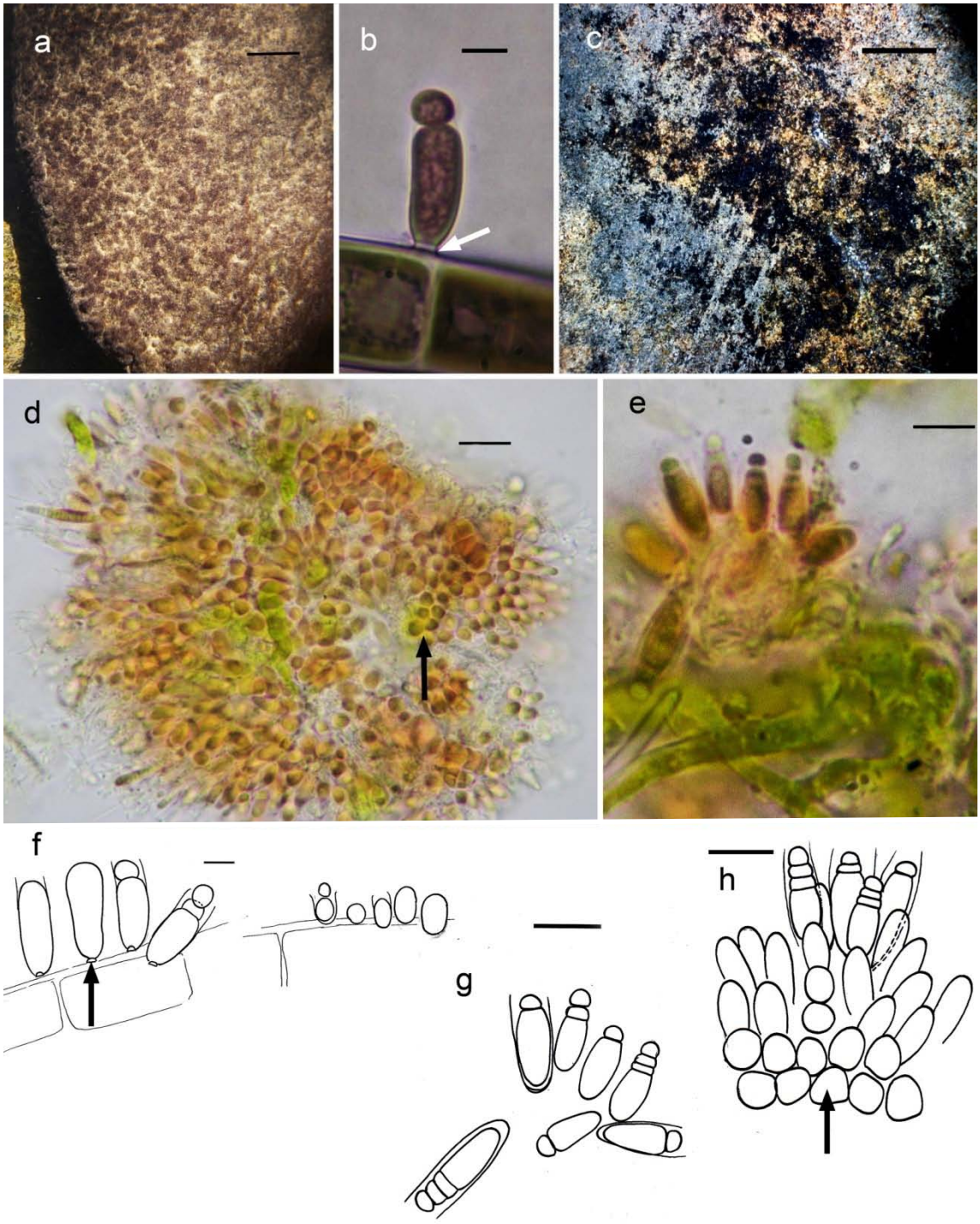

Figure 3.1: ***Chamaesiphon amethystinus***: **a**, crusts of *Batrachospermum* sp. on which epiphytic *C. amethystinus* was found; **b**, solitary cell with distinct basal pad (arrow), epiphytic on *Batrachospermum;* **f,** cell with rounded apex, blunt base and distinct basal pad (arrow). ***Chamaesiphon*** cf. ***brittanicus***: **c**, blackish-brown crust*;* **d**, top view of the colony showing polygonal outline of cells (arrow); **e**, irregularly arranged cells in the upper part of the colony; **g**, cells with 1-3 exospores; **h,** cross-section polygonal view from top (arrow). Scale bars: 10 µm for b, d-h; 5 cm for a, c.

Chamaesiphon cf. ***britannicus***(Fritsch) Komárek et Anagnostidis 1995
Komárek and Anagnostidis (1999): p389, fig. 505 (p388)

Description: Field specimens form blackish-brown crusts (Fig. 3.1c) which comprise colonies of 1-3 layers of densely aggregated cells. Layers are parallel, long axes of the cells are perpendicular to the substratum, upper part with more random arrangement of elongated cells, basal part with densely packed cells more or less polygonal in top view (Figs. 3.1d, g). Cells blue-green, cylindrical, 2.5-3.0 µm wide, 6.0-9.0 µm long, only occasionally narrowing towards the base. Cell content uniform. Exospores 1-3, spherical sometimes hemispherical and slightly flattened, 1.5-2.0 µm in diam. Sheath thin, firm, hyaline, visible in areas with lesser cell density (Fig. 3.1e, g).

Occurrence: Epilithic in unshaded third order stream.

Remarks: Specimens differ from *C. britannicus* (Komárek and Anagnostidis, 1999) in smaller cell dimensions and production of more exospores. The cell shape, including basal shape in cross-section, and aggregation to form colonies suggest that it is close to this species.

Chamaesiphon britannicus has been collected from flowing and stagnant clear waters in Europe as epiphytes on filamentous cyanobacteria (Komárek and Anagnostidis, 1999).

Chamaesiphon confervicolus *var*. ***confervicolus*** A Braun in Rabenhorst 1865
Komárek and Anagnostidis (1999): p381, fig. 495 (p.382)

Description: Field specimens attached to sporophyte-stage of the filamentous rhodophyte *Batrachospermum* (Fig 3.1a). Cells blue-green, club-shaped, densely aggregated in groups or sometimes solitary, individually attached to the substratum (Fig. 3.2a), 2.0-7.8 µm wide, 23.0-60.0 µm long, broadly rounded at the apex, attenuated towards the obtuse conical base, sometimes slightly withdrawn from the base of the pseudovagina. Cell content uniform. Exospores 1-4, spherical, 3.1-6.2 µm in diam. (Figs. 3.2b, f). Pseudovagina thin, hyaline, sometimes slightly widened towards the base or attenuated to a short stipe-like base (Fig. 3.2f).

Occurrence: Epilithic in shaded second order stream.

Remarks: Cell dimension is within the lower end of the range given for *C. confervicolus* var. *confervicolus* (Komárek and Anagnostidis, 1999).

Chamaesiphon confervicolus var. *confervicolus* colonies have previously been recorded as epiphytes on filamentous chlorophytes and rhodophytes in stagnant and flowing unpolluted waters, particularly in mountains from temperate to subpolar regions (Komárek and Anagnostidis, 1999).

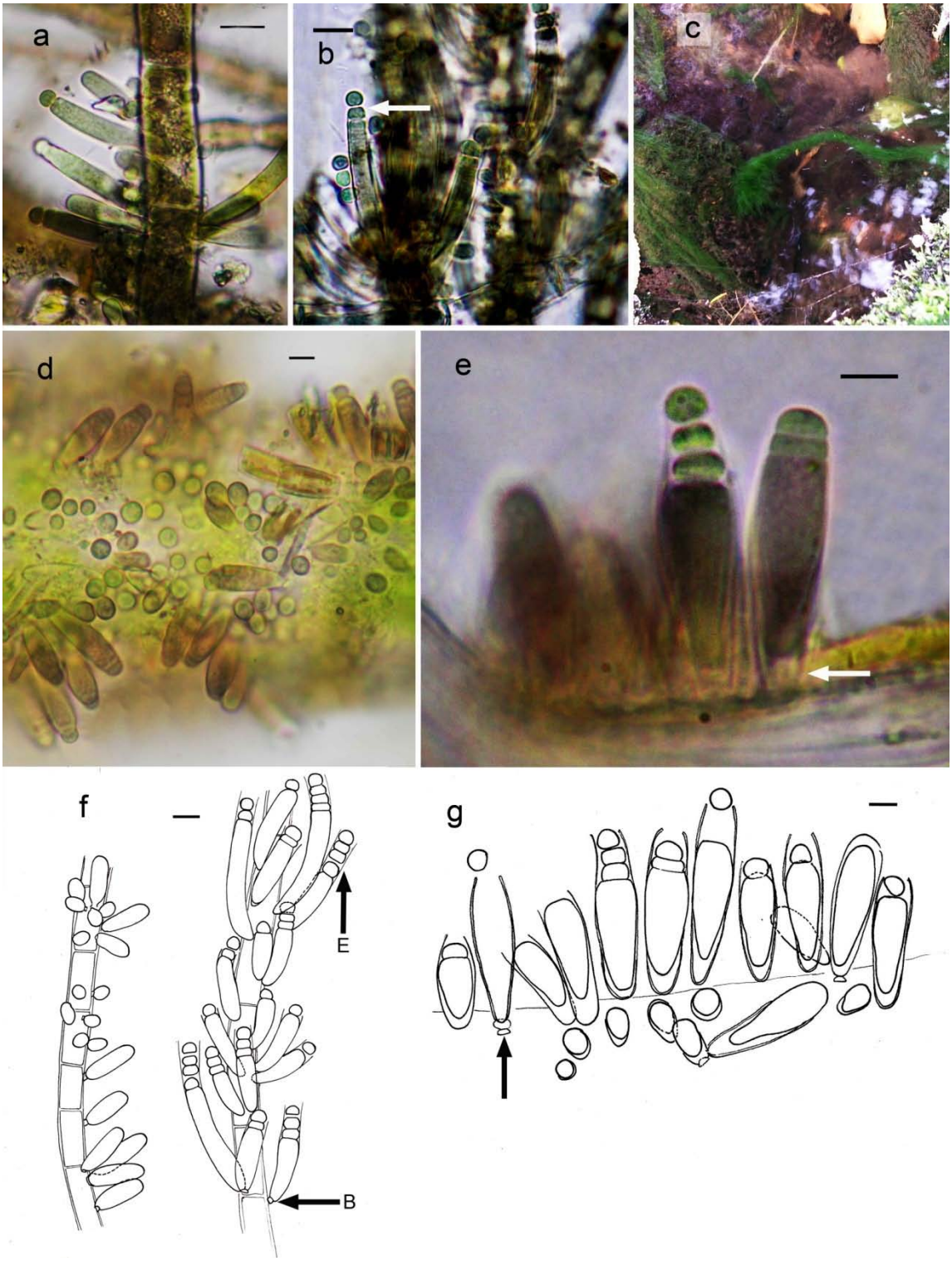

Figure 3.2: ***Chamaesiphon confervicolus*** var. ***confervicolus***: **a,** club-shaped cells individually attached to a filament of *Batrachospermum*; **b,** dense aggregation of cells with 1-3 exospores at the apex (arrow); **f,** large cells with 1-4 exospores (arrow E) and short stipe-like basal pads (arrow, B). ***Chamaesiphon incrustans***: **c,** filaments of *Cladophora* on which cells attach*;* **d**, dense aggregation of cells*;* **e,** formation of 2-3 exospores at cell apices, cells withdrawn from the sheath (arrow) ; **g,** cells withdrawn from bases of pseudovaginae some of which have basal pads (arrow). Scale bars: 10 µm for a, b, d – g; 1 cm for c.

Chamaesiphon incrustans Grunow in Rabenhorst 1895
Komárek and Anagnostidis (1999): p381, fig. 492-493 (p380)

Description: Field specimens attached to filamentous green alga *Cladophora* (Fig. 3.2c). Cells pale blue- green, somewhat cylindrical, densely aggregated, rarely solitary, individually attached to the substratum (Fig. 3.2d), 6.0-5.0 µm wide, 6.0-24.0 µm long , rounded at the apex, slightly conically narrowed at the base, sometimes slightly withdrawn from the base of the pseudovagina (Fig. 3.2e, g). Cell content uniform. Exospores 1-3, in a row at the apex of the mother cell, spherical, 3.0-4.0 µm in diam. (Fig. 3.2e). Pseudovagina distinct, thin, firm, hyaline, sometimes with a small basal pad (Fig. 3.2g).

Occurrence: Epiphytic in unshaded second order stream.

Remarks: Specimens fit the description of *Chamaesiphon incrustans.*

Previous records were from unpolluted flowing waters, particularly in mountainous areas of temperate to subpolar regions, as epiphytes on filamentous chlorophytes and rhodophytes (Komárek and Anagnostidis, 1999).

Chamaesiphon subglobosus (Rostafinski) Lemmermann 1910
Komárek and Anagnostidis (1999): p390, fig. 487 (p374), fig. 509 (p391)

Description: Field specimens are minor components of dark purple, approximately 0.5 mm diam., hemispherical colonies, densely aggregated to form crusts on cobbles (Fig. 3.3a). Cells blue- green, spherical, oval, 2.5-3.7 µm wide, 2.5-4.4 µm long, aggregated in rows, forming small irregular colonies (Figs. 3.3b, e). Cell content uniform. Exospore single, spherical sometimes hemispherical and slightly flattened, 1.9 µm in diam. (Fig. 3.3b, e). Sheath communal, indistinct, diffluent and hyaline.

Occurrence: Epilithic in unshaded second order stream.

Remarks: Specimens differ from *C. subglobosus* (Komárek and Anagnostidis, 1999) in the absence of granules. Granulation in cells can be very variable and highly dependent on the environment (Whitton, 2011). *Chamaesiphon subglobosus* is placed under the subgenus *Godlewskia* based on pseudofilament-like aggregations in which exospores remain attached to the communal sheath, sometimes forming rows or layers in the substratum (Komárek and Anagnostidis, 1999)

Previous records were from stagnant and running oligotrophic to mesotrophic, unpolluted waters, streams, waterfalls and lake litoral, mainly from central Europe. Mode of attachment can be epilithic and epiphytic on mosses and algae (Komárek and Anagnostidis, 1999).

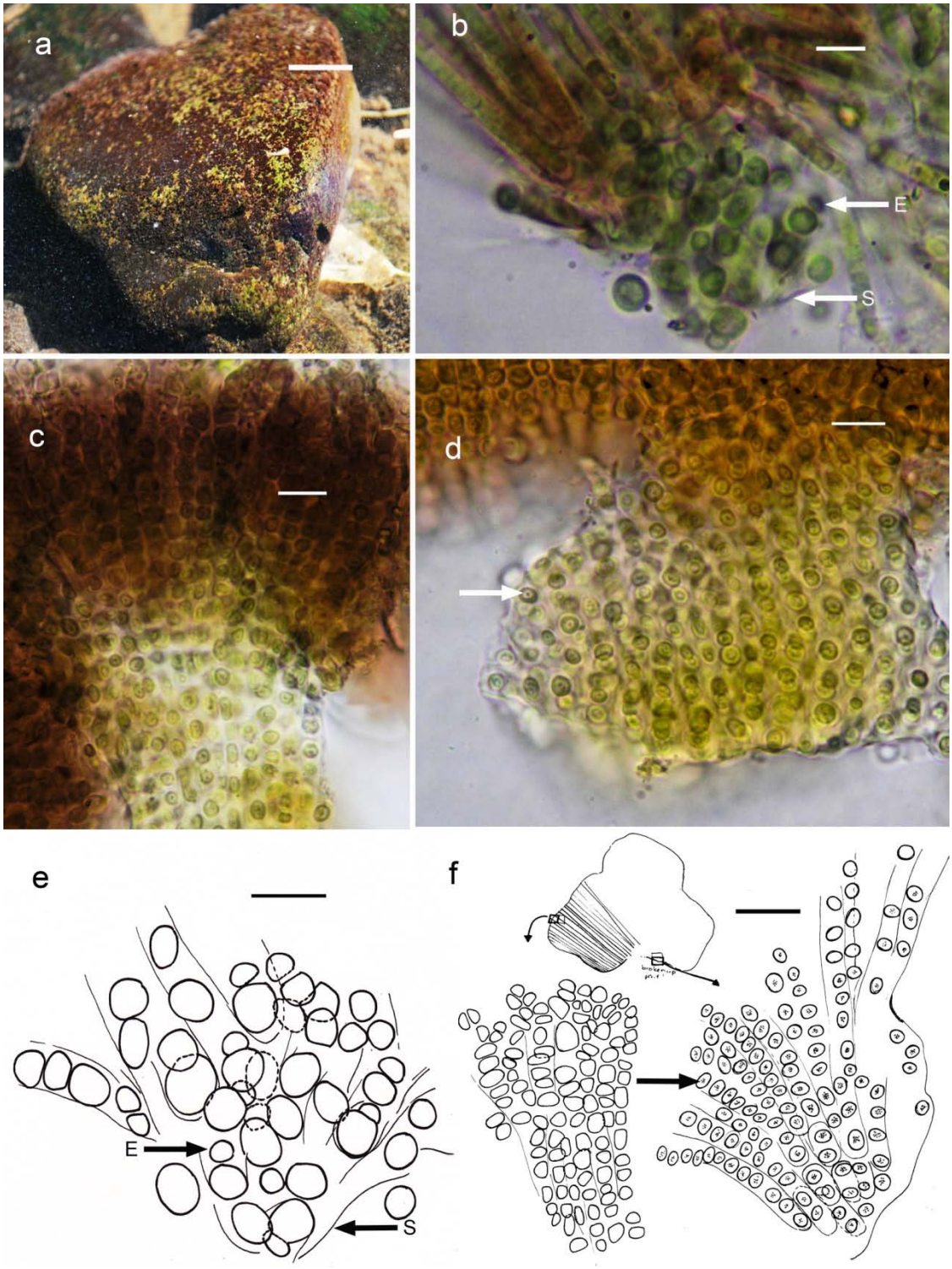

Figure 3.3: ***Chamaesiphon subglobosus***: **a,** dark purple, small, hemispherical colonies densely aggregated to form a crust on cobble; **b, e,** aggregation of cells in rows with exospores at the apex (arrow E) within diffluent communal sheath (arrow S). ***Chlorogloea*** cf. ***microcystoides***: **c,** cells densely packed at the periphery of a colony; **d**, communal sheath pigmented yellow, cell content granulated (arrow)*;* **f,** different cell arrangements within a colony. Scale bars: 5 µm for b-f; 2 cm for a.

Family **Entophysalidaceae**

Chlorogloea cf. ***microcystoides*** Geitler 1925
Komárek and Anagnostidis (1999): p329, fig. 434 (p327). Whitton (2011): p50

Description: Field specimens are minor components of crusts as for *Chamaesiphon subglobosus* (Fig. 3.3a). Cells blue- green arranged in radial rows, densely packed in the peripheral regions of the colony (Fig. 3.3c), sometimes distant from one another (Fig. 3.3f), 2.0-4.0 µm in diam., spherical, or ellipsoidal to slightly polygonal rounded (Fig. 3.3f). Cell content uniform, occasionally with small granules. Sheath communal but sometimes enveloping groups of cells, distinct, firm, colourless to pale yellow-brown (Fig. 3.3d).

Occurrence: Epilithic in unshaded second order stream.

Remarks: Specimens differ from *C. microcystoides* (Komárek and Anagnostidis, 1999) in wider cell dimensions and yellow-brown sheath pigmentation. A similar morphospecies from the British Isles resembles the present specimens (Whitton, 2011). Both were collected from well-illuminated sites.

Previous records were from stones in shallow waters at the edges of streams and lakes or in very moist adjacent areas (Whitton, 2011).

Family **Hydrococcaceae**

Cyanodermatium fluminense (Fritsch) Komárek et Anagnostidis 1995
Komárek and Anagnostidis (1999): p353, fig. 464 (p352). Whitton (2011): p57

Description: Field specimens are frequent components of crusts as for *Chamaesiphon subglobosus* (Fig. 3.3a). Growths comprise numerous, densely aggregated pseudofilaments (Fig. 3.4a, e). Cells pale blue- green, 2.0-3.5 (4.0) µm wide, 3.0-5.0 µm long, irregularly rounded, sometimes slightly cylindrical or isodiametric with slightly larger cells distally. Cell content uniform. Sheath communal, distinct, thin, firm.

Occurrence: Epilithic in unshaded second order stream

Remarks: Cell width, length, orientation and pigmentation suggest *C. fluminense* (Komárek and Anagnostidis 1999). The occurrence of individual sheaths within the communal mucilage was observed by Whitton (2011) in his specimens from the British Isles.

First described by Fritsch (1929). Other records were from similar habitat as the present collection (Komárek and Anagnostidis 1999; Whitton, 2011).

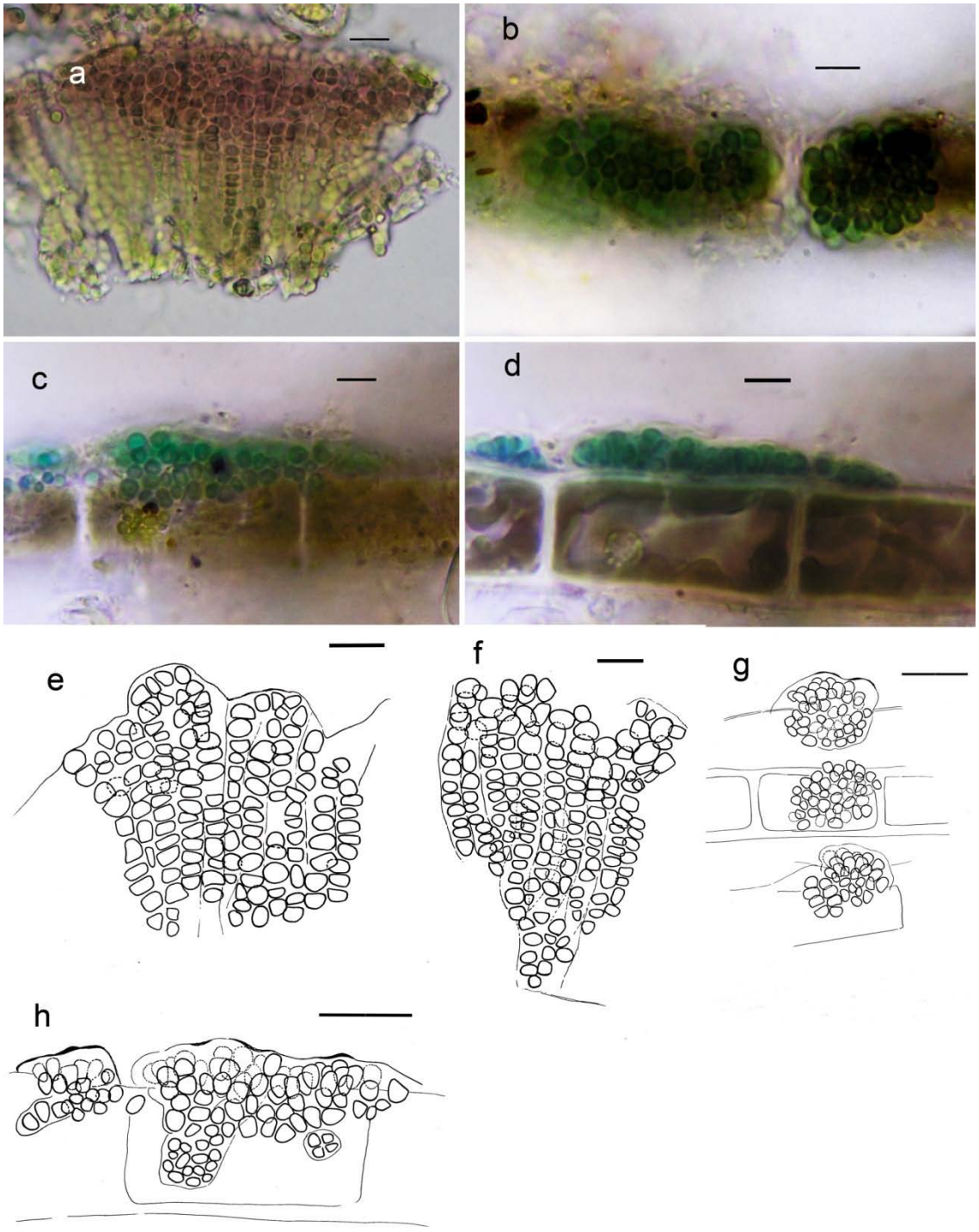

Figure 3.4: ***Cyanodermatium fluminense***: **a,** densely aggregated cells; **e,** cells forming pseudofilaments. ***Cyanodermatium*** sp.: **b,** bright blue-green cells epiphytic on *Batrachospermum*; **c,** cells forming indistinct rows; **d,** distinctly larger cells towards the colony margin; **g,** small colonies; **h**, variation in cell dimensions. ***Cyanodermatium*** cf. ***gigas***: **f,** numerous pseudofilaments with larger cells towards the margin. Scale bars: 5 µm for a-h.

Cyanodermatium cf. ***gigas*** (Geitler) Komárek et Anagnostidis 1995
Komárek and Anagnostidis (1999): p353, fig. 467 (p352)

Description: Field specimens are minor components of crusts as for *Chamaesiphon subglobosus* (Fig. 3.3a). Cells blue-green, densely aggregated in radial rows, 2.5-5.0 µm wide, 3.8-7.5 µm long , elongated rounded, sometimes slightly cylindrical or spherical with larger cells towards the margin (Fig. 3.4f). Cell content uniform. Sheath communal, distinct, thin, firm, hyaline sometimes with distinct individual sheath around each pseudofilments.

Occurrence: Epilithic in unshaded second order stream.

Remarks: Cell width and length is the major characteristic taken into account for placement in *C. gigas* (Komárek and Anagnostidis 1999), a morphotype from the tropics. The sample clearly exceeded cell size of European *C. fluminense* which is only (2)3.5-5 x 2-3.5 µm. The current specimens have shorter cells than *C. gigas* in which length is 5-8 (12) µm (Komárek and Anagnostidis, 1999).

Cyanodermatium gigas is recorded on stones in streams in Sumatra (Komárek and Anagnostidis 1999). Poorly known genus worldwide.

Cyanodermatium sp.
Komárek and Anagnostidis (1999): p351

Description: Field specimens form colonies, attached to *Batrachospermum* sporophyte (Fig. 3.1a). Colonies comprise 2-3 layers of cells (Fig. 3.4b) that form numerous radial rows resembling pseudofilaments (Fig. 3.4c). Cells bright blue-green, densely aggregated especially at the periphery of the colony (Fig. 3.4b), 1.0-1.5 (2.0) µm wide, (1.5) 2.5-3.0 µm long, irregular in shape, sometimes rounded or spherical or hemispherical (Fig. 3.4g), with distinctly larger cells towards the margin (Figs. 4d, h). Cell content uniform. Sheath communal, distinct, generally thin, thicker only at the apical part of the colony, firm, hyaline.

Occurrence: Epiphytic in shaded third order stream.

Remarks: Colony appearance with rows of densely packed pseudofilaments suggests placement in *Cyanodermatium*. Cell dimensions and mode of attachment of the current specimens differ from species described in Komárek and Anagnostidis (1999).

Hydrococcus cf. ***rivularis*** Kützing 1833
Komárek and Anagnostidis (1999): p355, fig. 469 (p356)

Description: Field specimens form flat colonies, almost circular in outline, attached to the substratum. Cells blue-green, 2.5-4.4 µm in diam., polygonal rounded, sometimes slightly cylindrical or isodiametric in the middle of the colony, more hemispherical towards the margin, densely

aggregated in 1-2 layers, forming radial pseudofilaments (Fig. 3.5a). Cell content uniform, occasionally with small granules (Fig. 3.5b). Sheath communal, distinct, thin, firm, hyaline, occasionally individual sheath occurs cell clusters.

Occurrence: Epilithic in unshaded second order stream.

Remarks: Differs from *H. rivularis* (Komárek and Anagnostidis, 1999) in marginal cells being neither elongated nor arcuated. Cell diam. is at the lower end of the given range. Cell density is higher at the periphery of the colony.

Hydrococcus rivularis grows on stones, mosses, water plants, wooden substrata and other algae in rapid, clear, cold, mountain streams, rivers and waterfalls (Komárek and Anagnostidis, 1999; Whitton, 2011).

Placoma regulare Broady et Ingerfeld 1991
Broady & Ingerfeld (1991): p547-555, fig. 7-14 (p550)

Description: Field specimens form purplish colonies (Fig. 3.5c), up to 5mm diam., consisting of a membranous sheet folded into a somewhat cerebriform, hollow sac; young colonies solid, up to 0.1 mm diam. Cells spherical, blue-green, arranged in pseudofilaments (Fig. 5d), up to 3 layers thick, outer cells 2.0 µm in diam., innermost cell up to 20 µm in diam. Sheath thick, firm, hyaline.

Cultures on agarised medium form colonies up to 3 mm diam., black, irregular in shape with undulated surface. Cells spherical to cuboid, purplish, arranged in pseudofilaments , radiating from the center of the colony (Fig. 3.5h), up to 3 layers thick; outer cells 2.0 µm in diam., densely aggregated, dividing to form small clusters with up to 4 cells; innermost cells 10 µm in diam. (Fig. 3.5g). Sheath communal, thick, yellowish, occasionally lamellated (Fig. 5i).

Occurrence: Epilithic in unshaded second order stream.

Remarks: Colony size and innermost cell dimensions fall within the lower end of the recorded range (Broady and Ingerfeld, 1991). Colony appearance and cell arrangement changed markedly in cultures. Sheath appears yellow-brown indicating the presence of the pigment scytonemin which has been commonly observed in slow-growing cultures (Whitton, 2011).

Previously recorded from rocks and bryophytes in small streams in New Zealand (Broady and Ingerfeld, 1991).

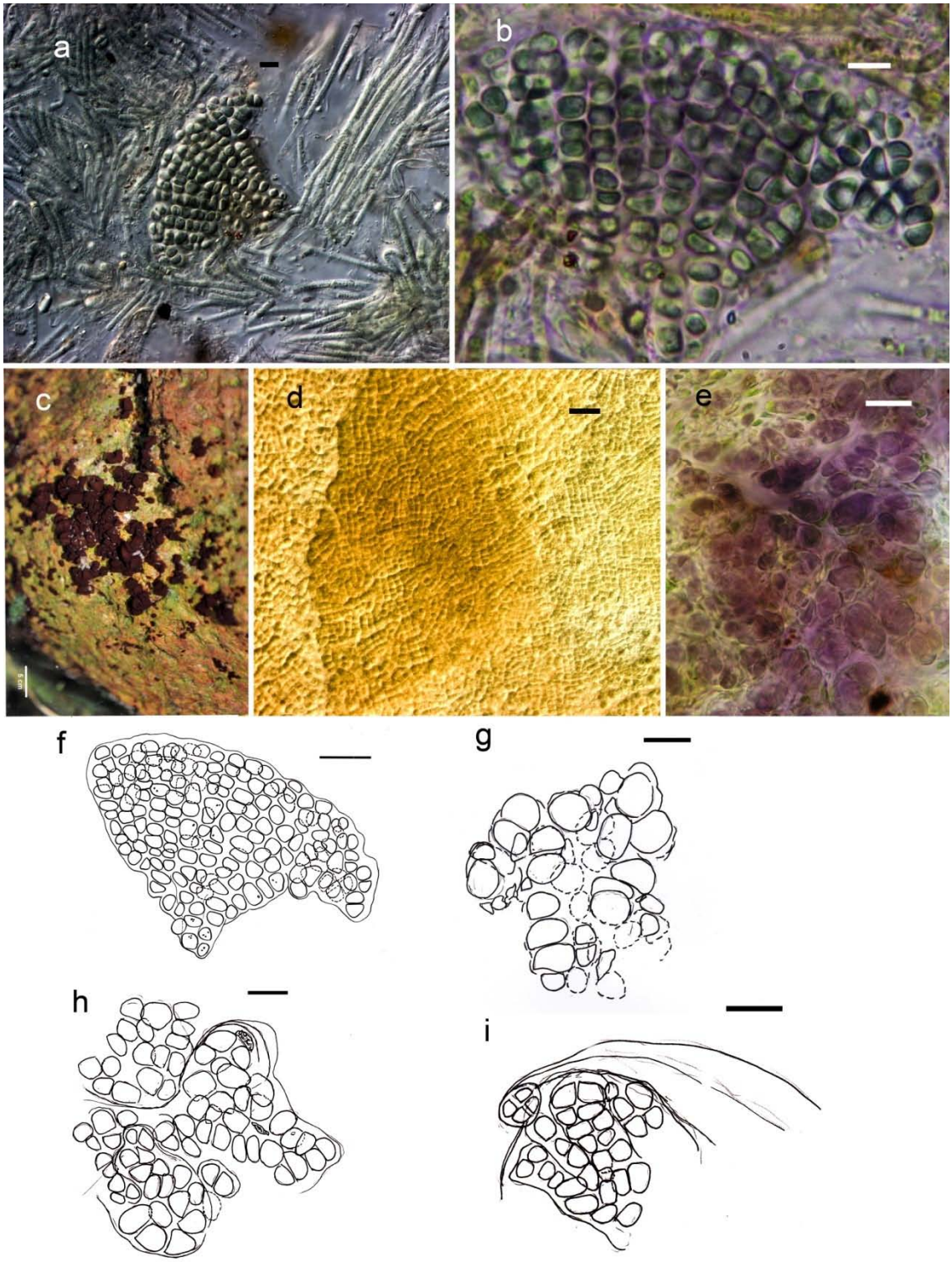

Figure 3.5: ***Hydrococcus*** cf. ***rivularis*****: a,** pseudofilaments; **b,** cell content occasionally with small granules; **f,** densely aggregated cells. ***Placoma regulare***; field specimens, **c,** purplish gelatinous epilithic colonies; **d,** cell arrangement; cultures, **e,** cell morphology and pigmentation; **g,** larger cell dimensions in centre of colony; **h,** cell arrangement towards the colony periphery; **i**, cell division at the periphery, enclosed in a thick sheath . Scale bars: 5 µm for a, b, d-i; 5 mm for c.

Family **Hyellaceae**

Pleurocapsa cf. ***minor*** Hansgirg 1891
Komárek and Anagnostidis (1999): p465, fig.616 (p466), fig. 618 (p470)

Description: Field specimens form flat colonies, attached to the substratum, composed of short pseudofilaments, 7.0-25.0 µm long, sometimes divaricate, 1-2 cell layers thick. Cells purplish or blue-green (Fig., 3.6a, b), 2.0-6.0 µm wide, barrel-shaped to irregularly spherical or asymmetrical, with slight elongation at the margin of the colony (Fig. 3.6b). Cell content uniform. Baeocytes spherical, 1.0-2.5 µm diam. (Fig. 3.6g), formed by any cell, up to 9 per cell. Sheath communal, distinct, thin and firm.

Occurrence: Epilithic in unshaded fourth order stream.

Remarks: Specimens differ from *P. minor* (Komárek and Anagnostidis 1999) in smaller numbers of baeocytes produced in a cell (8-32) and lack of distinct elongation of apical cell.

Previous records from streams, rivers and edges of lakes usually on or among calcareous substrata (Whitton, 2011).

Pleurocapsa sp.
Komárek and Anagnostidis (1999): p464

Description: Field specimens form flat colonies, attached to the substratum, composed of erect, parallel, dense, short, heteropolar pseudofilaments (Fig. 3.6c, d). Cells generally yellowish-brown, dark blackish-brown only towards the apex of pseudofilaments, 3.8-6.9 µm wide, 4.4-7.5 µm long, isodiametric, sub-spherical, rounded and elongated towards the apex, apical cell broadly rounded (Fig. 3.6e,h). Baeocyte production not observed. Sheath individual, thicker towards apex of pseudofilaments, yellowish-brown and firm.

Occurrence: Epilithic in unshaded fourth order stream.

Remarks: Occurrence of heteropolar pseudofilaments suggests placement in Hyellaceae Borzi 1914. Pseudofilaments with distinct rows of cells suggest subfamily Hyelloideae. The lack of distinct spaces between cells in pseudofilaments that grow over the substratum suggests *Pleurocapsa*.

Only two freshwater morphospecies, *P. aurantiaca* Geitler 1932 (p468, fig. 612) and *P. fusca* Godward 1937 (p468, fig. 613) have pigmented sheaths. The present material did not conform to the two nominal varieties.

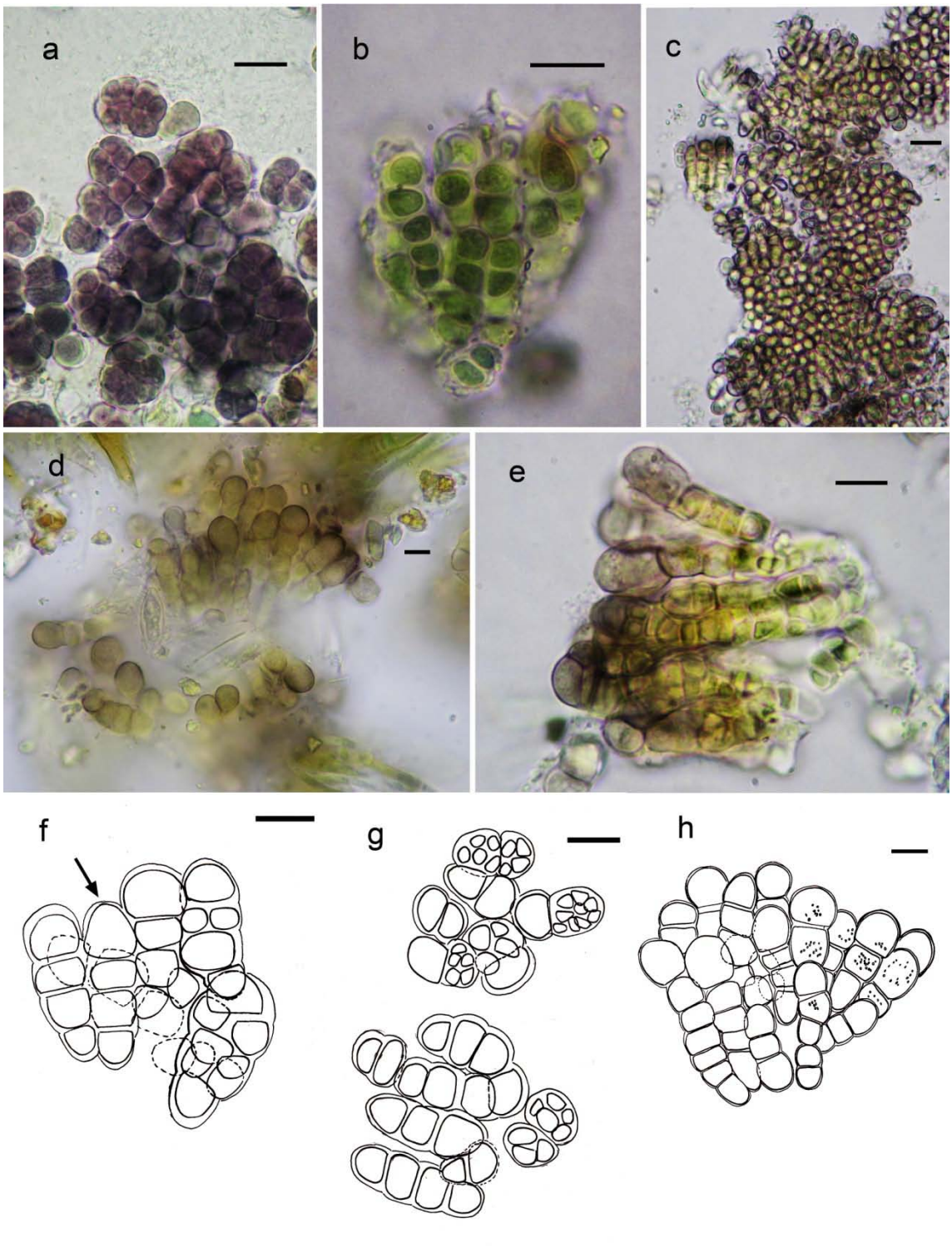

Figure 3.6: ***Pleurocapsa*** cf. ***minor***: **a,** cells forming short pseudofilaments; **b,** apical cells with slight elongation; **f,** apical cell broadly rounded occasionally slightly elongated (arrow); **g,** baeocyte formation in any cell, up to 9 per cell. ***Pleurocapsa*** sp.: **c,** colony with very dense aggregations of pseudofilaments; **d,** erect pseudofilaments; **e,** pseudofilaments changing colour from blue-green at base, through brown to purplish-brown towards apex; **h**, heteropolar pseudofilaments with apical cells wider than basal cells. Scale bars: 5 µm for a, b, d-h; 10 µm for b.

Radaisia sp.
Komárek and Anagnostidis (1999): p448

Description: Field specimens are minor components of crust as for *Chamaesiphon subglobosus*. Pseudofilaments 14-33 µm long, sometimes divaricate (Fig. 3.7a, b). Cells blue-green, 2.5-7.5 µm diam., more or less isodiametric or irregularly spherical. Distinctly larger cells at colony margin form baeocytes. Baeocytes blue-green, spherical, 1.3-3.1 diam., arise by successive cell division (Fig. 3.7g). Sheath communal, distinct, thin and firm.

Occurrence: Epilithic in unshaded second order stream.

Remarks: Diagnostic characteristics of *Radaisia* Sauvageau (1895) are possession of pseudofilaments with only apical cells producing baeocytes. *Radaisia confluen*s Gardner (1927) and *R. willei* Gardner (1927) have similar ecology but cell dimensions for both differ from the present material.

Family **Merismopediaceae**

Merismopedia punctata Meyen 1839
Komárek and Anagnostidis (1999): p175, fig. 222 (p178)

Description: Colonies in agarised culture blue-green, flat, with one cell layer of 16 to more than 60 cells arranged in more or less regular rows (Fig. 3.7c, h). Cells pale blue-green, spherical to widely oval to hemispherical after division, 2.5-3.0 µm diam. (Fig., 3.7d). Mucilage homogeneous, thin, firm and hyaline.

Remarks: Specimens were recorded only from cultures inoculated with crust material. Colony morphology and cell dimensions fall within the given range for *M. punctata* (Komárek and Anagnostidis, 1999).

Previous distribution data suggest the species is planktonic and metaphytic in mesotrophic freshwater habitats. Similar morphospecies are often recorded from brackish water worldwide (Komárek and Anagnostidis 1999).

Family **Synechococcaceae**

Aphanothece clathrata W. et G.S. West 1906
Komárek and Anagnostidis (1999): p75, fig.58 (p76)

Description: Colonies in agarised culture blue-green, irregular in outline (Fig. 3.7i), made up of densely aggregated cells (Fig. 3.7e). Cells bright blue-green, rod-shaped, sometimes slightly curved (Fig. 3.7f), 1.0 µm wide, 2.0-4.0 µm long, without gas vesicles. Mucilage homogeneous and hyaline.

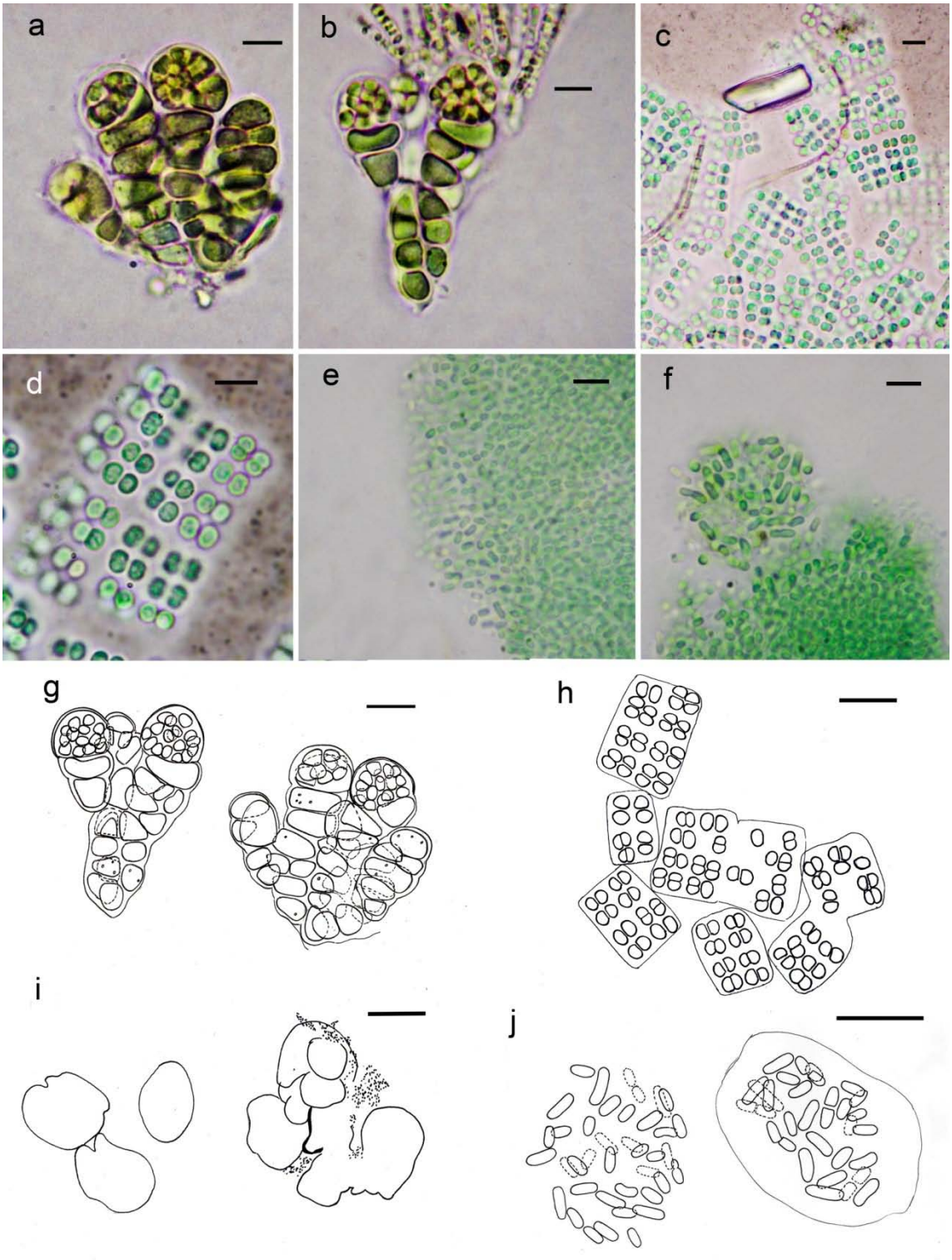

Figure 3.7: ***Radaisa*** **sp.: a, g,** short pseudofilaments, baeocyte formation in distinctly larger apical cells; **b,** branched pseudofilaments. ***Merismopedia punctata***: **c,** plate-shaped colonies; **d,** cells bound within common mucilage; **h**, colonies with 16-30 loosely arranged cells. ***Aphanothece clathrata***: **e,** densely aggregated cells within a common mucilage; **f, j,** rod-shaped cells; **i,** irregular colony outline. Scale bars: 10 µm for a, b, g-j; 5 µm for c-f.

Remarks: Specimens were recorded only from cultures inoculated with crust material. Conforms closely to the description given by Komárek and Anagnostidis (1999).

Previously recorded as phytoplankton of mesotrophic and eutrophic lakes, ponds, pools and rivers (Komárek and Anagnostidis, 1999).

Family **Xenococcaceae**

Xenococcus sp.
Komárek and Anagnostidis (1999): p428, 433, fig. 570 (p434)

Description: Colonies of field specimens epiphytic on the sporophyte of the filamentous rhodophyte Batrachospermum (Fig. 3.8a, e), with cells packed very closely in one layer, sometimes indistinctly forming short, irregular rows. Cells dark blue-green to purplish, 5.0-16.3 µm diam., broadly oval, spherical to almost cylindrical; in larger cells sometimes very slightly narrowed towards the base. Baeocytes irregular in shape, 2.5-3.8 µm diam., up to 11 in a sporangium (Fig. 3.8b, f). Sheath around individual cells, distinct, thin, firm, hyaline.

Occurrence: Epiphytic in shaded third order stream.

Remarks: Only two freshwater morphospecies have been thoroughly discussed by Komárek and Anagnostidis (1999): *X. minimus* Geitler (1922) and *X. gr*acilis Lemmermann (1898). Both are epiphytic but with distinctly smaller cell dimensions (2.2 x ± 1.6 µm and 3.0-5.5 x 1.5-3.0 respectively) than Kaituna specimens. They list five more freshwater epiphytic morphospecies outside Europe. The illustration of *X. willei* superficially resembles the present material but cell dimensions are not given (Komárek and Anagnostidis, 1999, fig 570).

Xenotholos kerneri (Hansgirg) Gold-Morgan *et al.* 1994
Komárek and Anagnostidis (1999): p440, fig. 582. Whitton *et al.* (2011): p77, pl. 14J-N (p70)

Description: Colonies of field specimens epiphytic on colonies of *Placoma regulare* (Fig. 3.8c), form rounded colonies with 1-2 layers of densely aggregated cells in short irregular rows. Cells bright blue-green, 2.0-8.0 µm long, 2.5-6.0 µm wide; inner cells irregularly rounded, similar in size; outer cells broader, somewhat polygonal (Fig. 3.8g). Baeocytes not observed. Sheath distinct, thin, firm, hyaline, individual.

Specimens on agarised culture medium form irregular, purplish to greyish blue-green, more or less sarcinoidal cell aggregates (Fig. 3.8d). Cells 5.0-10.0 µm long, 2.5-7.5 µm wide and with colour pigmented sheath. Baeocytes not observed.

Occurrence: Epiphytic in partly shaded third order stream.

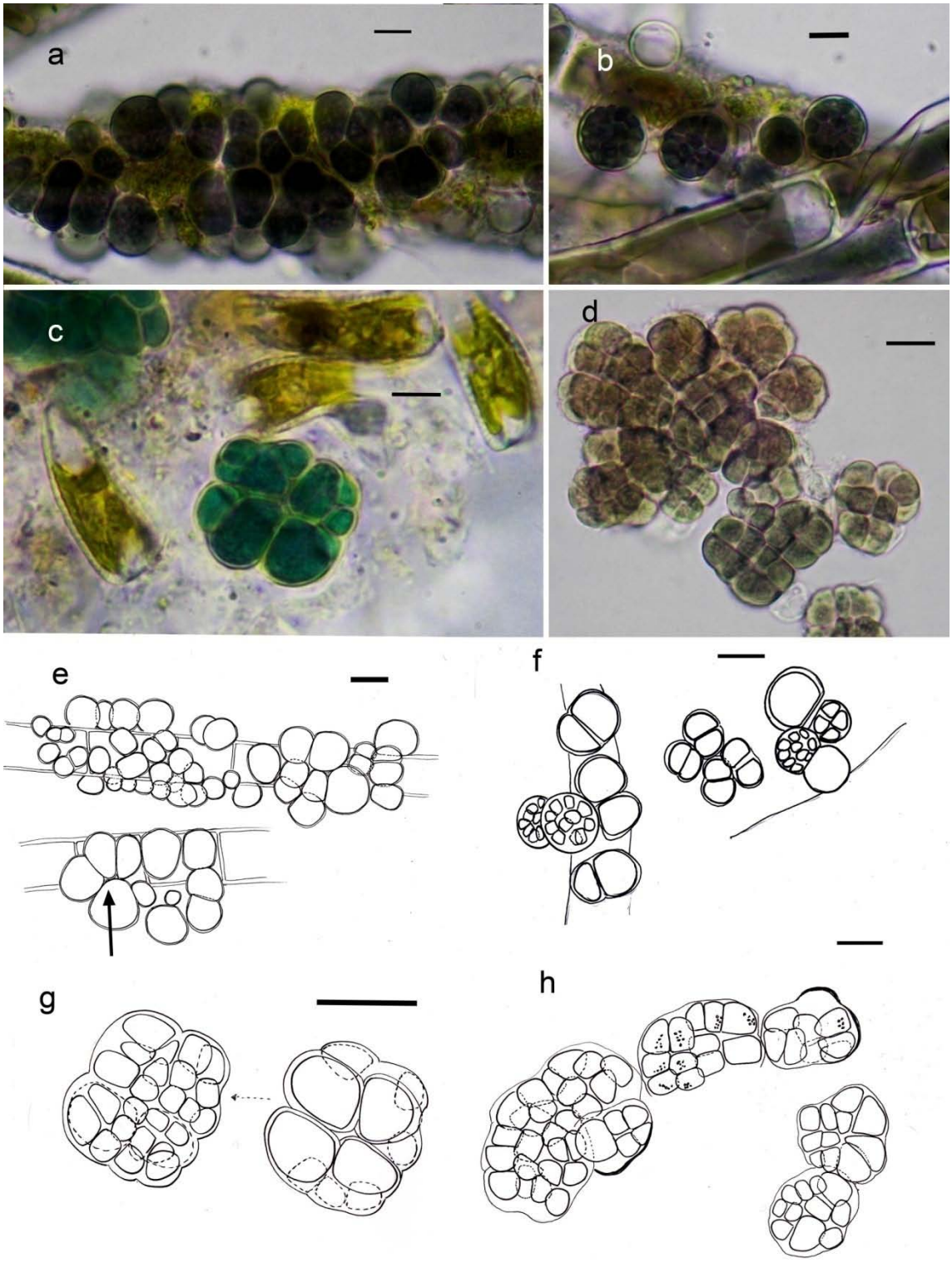

Figure 3.8: ***Xenococcus*** sp.: **a,** densely aggregated cells epiphytic on sporophyte of *Batrachospermum*; **b,** baeocyte formation; **e,** broadly oval cells, larger cells are slightly narrowed towards the base (arrow); **f,** baeocyte production by successive cell division. ***Xenotholos kerneri***: field specimens, **c**, a small colony; **g**, range in cell dimensions; cultures, **d,** cells pigmented purplish to greyish blue-green, more or less sarcinoidal cell aggregates; **h,** colony morphology with similar cell arrangement to that of field-collected colonies. Scale bars: 5 µm for a-f, h; 10 µm for g.

Remarks: Field specimens differ from *X. kerneri* (Komárek and Anagnostidis, 1999) in the absence of a thick, lamellated, pigmented sheath, however, these sheath characteristics have been observed in cultures.

Xenotholos kerneri has been recorded as an epiphyte on mosses and larger algae and as epilithon on stones in mountain streams, waterfalls and rivers (Komárek and Anagnostidis 1999).

Family **Pseudanabaenaceae**

Geitlerinema amphibium (Agardh ex Gomont) Anagnostidis 1989
Komárek and Anagnostidis (2005): p127, fig. 136. McGregor (2007): p22, pl. 1I, fig. 3D.

Description: Mats in the field, flat, bright blue-green, thin, slimy, and loosely coherent on silt (Fig. 3.9a). Trichomes pale blue-green, isopolar, long, straight or flexuous, 2.0 - 2.5 µm wide, not constricted at indistinct cross walls, lacking or with very slight attenuation at the ends, markedly motile (Fig. 3.9b, f). Cells longer than wide, 3.0-4.5 µm long, with 1 to 2 distinct cyanophycin granules on both sides of each cross wall. Apical cell rounded, sometimes slightly bent and attenuated. Sheath absent.

Colonies on agarised culture medium, bright blue-green mats, slimy, loosely coherent and patterned with swirls. Trichome morphology (Fig. 3.9c) similar to field material.

Occurrence: Mats growing at site 21 in the unshaded fourth order stream. Presence was only recorded on one occasion in early autumn 2010.

Remarks: Phenotypic characteristics are almost identical in field and cultured specimens. This contradicts the finding of Romo *et. al* (1993). Their cultured specimens, of field material identified as *G. amphibium*, had apical cells which were narrower and more curved. These features more resemble *G. ionicum* (Bory ex Gomont) Anagnostidis et Komárek 1988.

Geitlerinema amphibium has been recorded from streams, soils, stagnant waters (Komárek and Anagnostidis, 2005) and as phytoplankton in large lakes and reservoirs (McGregor, 2007). Records from brackish and marine environments are probably other morphospecies (Komárek and Anagnostidis, 2005).

Geitlerinema ionicum (Bory ex Gomont) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p124, fig. 129 (p125)

Descriptions: Field specimens not forming visible mats. Trichomes frequently observed entangled amongst other algae masses, bright blue-green, isopolar, long, straight or flexuous, narrow, 1.0-1.5 µm wide, not constricted at cross walls, slightly attenuated and bent at the ends, markedly motile (Fig. 3.9d). Cells longer than wide, 3.0-6.0 µm long, with 2-3 distinct cyanophycin granules at each cross-

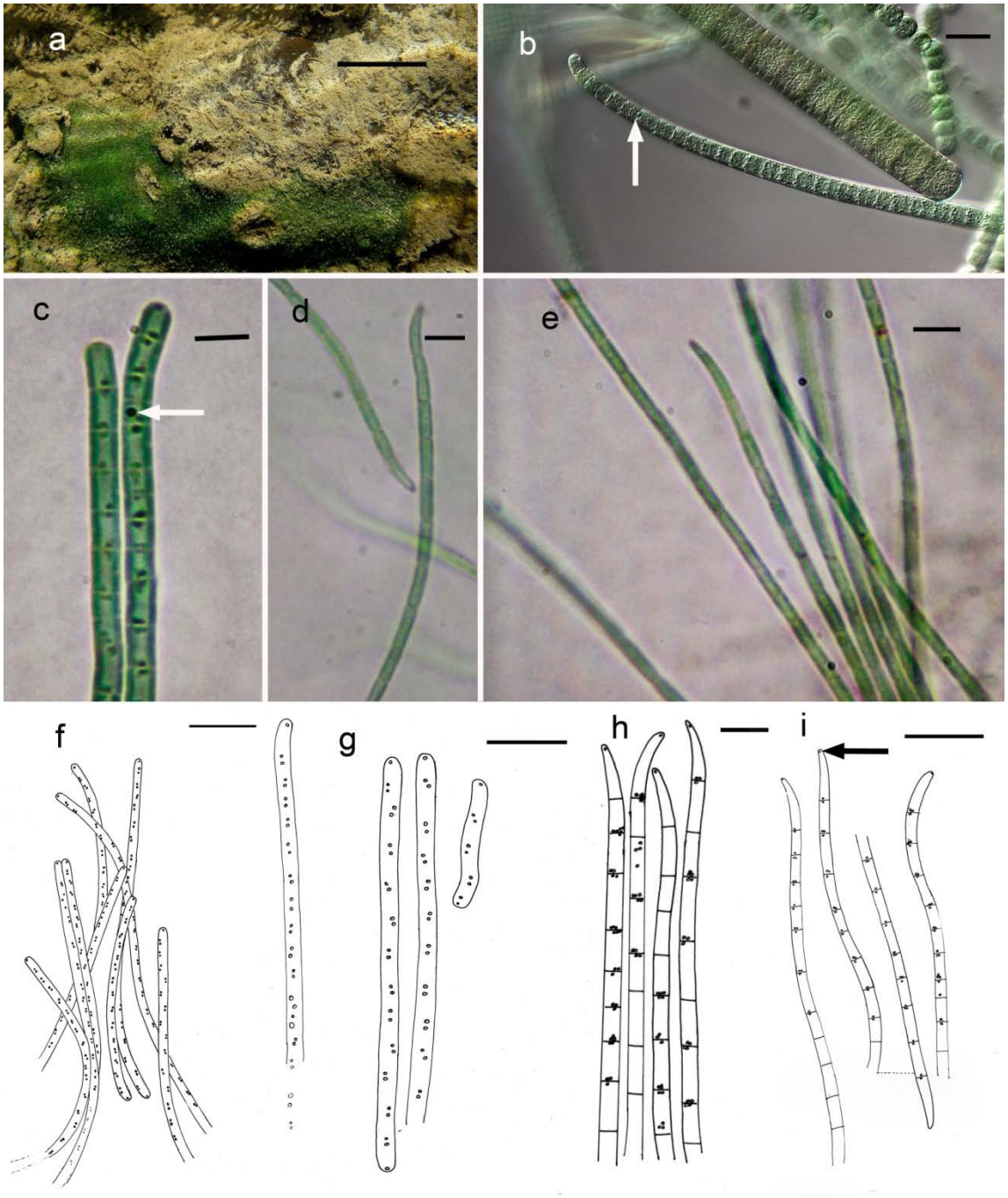

Figure 3.9: ***Geitlerinema amphibium*****:** field specimens**, a,** bright blue-green, thin, slimy mats**; b,** trichome morphology (arrow); **f,** long, flexous trichomes; cultures, **c,** cyanophycin granule at each side of each cross-wall (arrow) ; **g,** trichome morphology similar to field specimens. ***Geitlerinema ionicum*****:** field specimens, **d,** narrow trichome with bent apical cells; **h,** trichomes with cells longer than wide; cultures, **e,** trichome morphology; **i,** apical cell shape and granulation patterns. Scale bars: 5 µm for b-i; 2 cm for a.

wall. Apical cell elongated, bluntly pointed, bent, with a prominent apical cyanophycin granule. Sheath absent (Fig. 3.9h).

Colonies on agarised culture medium bright blue-green mats, thin, radiating from the point of inoculation. Trichomes identical to field material (Fig. 3.9e, i). Sheath thin, hyaline.

Occurrence: A common component amongst other algae masses in unshaded fourth order stream.

Remarks: Specimens fit Komárek and Anagnostidis (2005) species concept for *G. ionicum*. Apical cell shape and the presence of cyanophycin granules is a diacritical feature.

Previously described from freshwater in Turkey, waterfalls and rice-fields in Greece (Komárek and Anagnostidis 2005).

Heteroleibleinia fontana (Hansgirg) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p250, fig. 328 (p251)

Description: Field specimens are frequent components of crusts as for *Chamaesiphon* cf. *subglobosus* (Fig. 3.10a). Filaments pale blue-green, heteropolar with one end attach to the substratum, long, straight or flexuous, forming denseparallel clusters (Fig. 3.10b, f). Trichomes, 2.4-3.0 µm wide, constricted at cross-walls. Cells barrel-shaped, isodiametric or shorter than wide, 0.8-1.6 µm long. Apical cell obtuse rounded. Sheath thin, firm and hyaline.

Colonies on agarised culture medium purplish- black with circular outline. Filaments free-living, not attached to the agar surface. Trichomes resemble field specimens except slightly narrower, 2.0-2.5 µm wide (Fig. 3.10c, g). Sheath always observed, thin, firm, hyaline.

Occurrence: Epilithic in unshaded second order and partly shaded third order streams.

Remarks: Specimens differ from the description in Komárek and Anagnostidis (2005) only in having cell width at the narrower end of the given range. Filaments observed on agarised culture medium resemble *L. foveolarum* as they are not attached.

Previous collection in Czech Republic from similar a small stream (Komárek and Anagnostidis 2005).

Heteroleibleinia cf. ***kossinskajae*** (Elenkin) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p246, fig. 321 (p247)

Description: Field specimens are minor components of crusts as for *Chamaesiphon* cf. *subglobosus* (Fig. 3.10a). Filaments pale blue-green, heteropolar with one end attach to substratum, long, straight or slightly flexuous, densely aggregated (Fig. 3.11a). Trichomes 1.3-1.5 µm wide, slightly constricted at cross walls. Cells barrel-shaped, isodiametric or generally shorter than wide, 0.6-1.3 µm long apart from ones close to the apex which are longer than wide, 2.5-3.1 µm (Fig. 3.11e). Apical cell conically rounded, slightly attenuated and bent. Sheath thin, firm and hyaline.

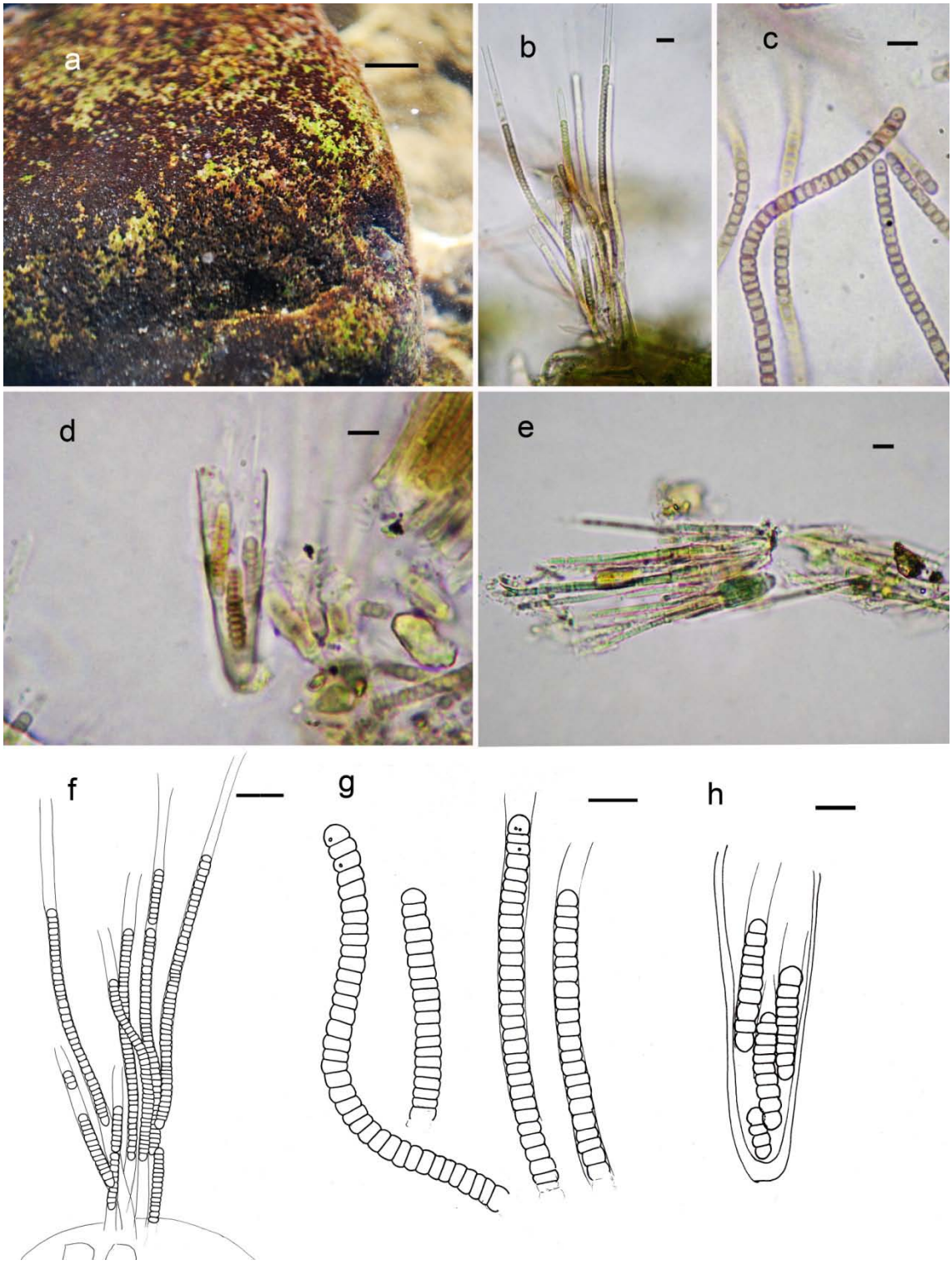

Figure 3.10: ***Heteroleibleinia fontana*****: a,** colonies dark purple, hemispherical, densely aggregated forming a crust; field specimens, **b, f,** filament morphology, heteropolar trichomes basally attached to the substratum; cultures, **c, g,** free living trichome morphology (unialgal); **d, e, h,** colony appearance with filaments within empty *Chamaesiphon* sheaths (mixed). Scale bars: 5 µm for b-h; 2cm for a.

Occurrence: Epilithic in unshaded second order stream.

Remarks: Specimens differ from *H. kossinskajae* (Komárek and Anagnostidis 2005) in having cells 2-3 times longer than wide. Trichome width is in the lower end of given range. Elongation of apical cell in the present specimen is marked.

Heteroleibleinia kossinskajae previously described as epiphytic on the filamentous green alga, *Cladophora* (Komárek and Anagnostidis 2005).

Heteroleibleinia cf. ***pusilla*** (Hansgirg) Compère 1985
Komárek and Anagnostidis (2005): p245, fig. 319 (p247)

Description: Field specimens are frequent components of crusts as for *Chamaesiphon* cf. *subglobosus* (Fig. 3.10a). Filaments pale blue-green, heteropolar with one end attach to substratum, short, usually less than 80 µm long, straight or rarely slightly curved, densely aggregated (Fig. 3.11b, c, f). Trichomes 1.0-1.5 µm wide, constricted at cross walls. Cells barrel-shaped, isodiametric , 1.0-1.5 µm long. Apical cell obtuse rounded, without a calyptra. Sheath thin, firm and hyaline.

Occurrence: Epilithic in unshaded second order stream.

Remarks: Specimens differ from *H. pusilla* (Komárek and Anagnostidis 2005) in being constricted at cross walls.

Heteroleibleinia pusilla has been recorded as an epilith in clear, flowing water in the Czech Republic, (Komárek and Anagnostidis 2005).

Heteroleibleinia cf. ***versicolor*** (Wartman) Gomont 1893
Komárek and Anagnostidis (2005): p252, fig. 330 (p251)

Description: Field specimens are minor components of crusts as for *Chamaesiphon* cf. *subglobosus* (Fig. 3.10a). Filaments pale blue-green, heteropolar with one end attach to the substratum, long, straight or slightly flexuous, solitary or aggregating in small groups (Fig. 3.11g), 5.6 µm wide. Trichomes 1.6-2.4 µm wide, constricted at cross walls. Cells barrel-shaped, almost isodiametric or shorter than wide, 0.8-1.6 µm long. Apical cell rounded. Sheath distinctly widened, without lamellation, firm and hyaline.

Occurrence: Epilithic in unshaded second order stream.

Remarks: Specimens differ from *H. versicolor* Komárek and Anagnostidis (2005) in sheaths lacking pigmentation and lamellation. Cell dimensions are at the lower end of the given range. *H. versicolor* is the only freshwater morphospecies in the genus with a prominent wide sheath.

Previous records from Europe, North America and West Indies with no description of collection sites. *Heteroleibleinia versicolor* is a rare and poorly known morphospecies (Komárek and Anagnostidis, 2005).

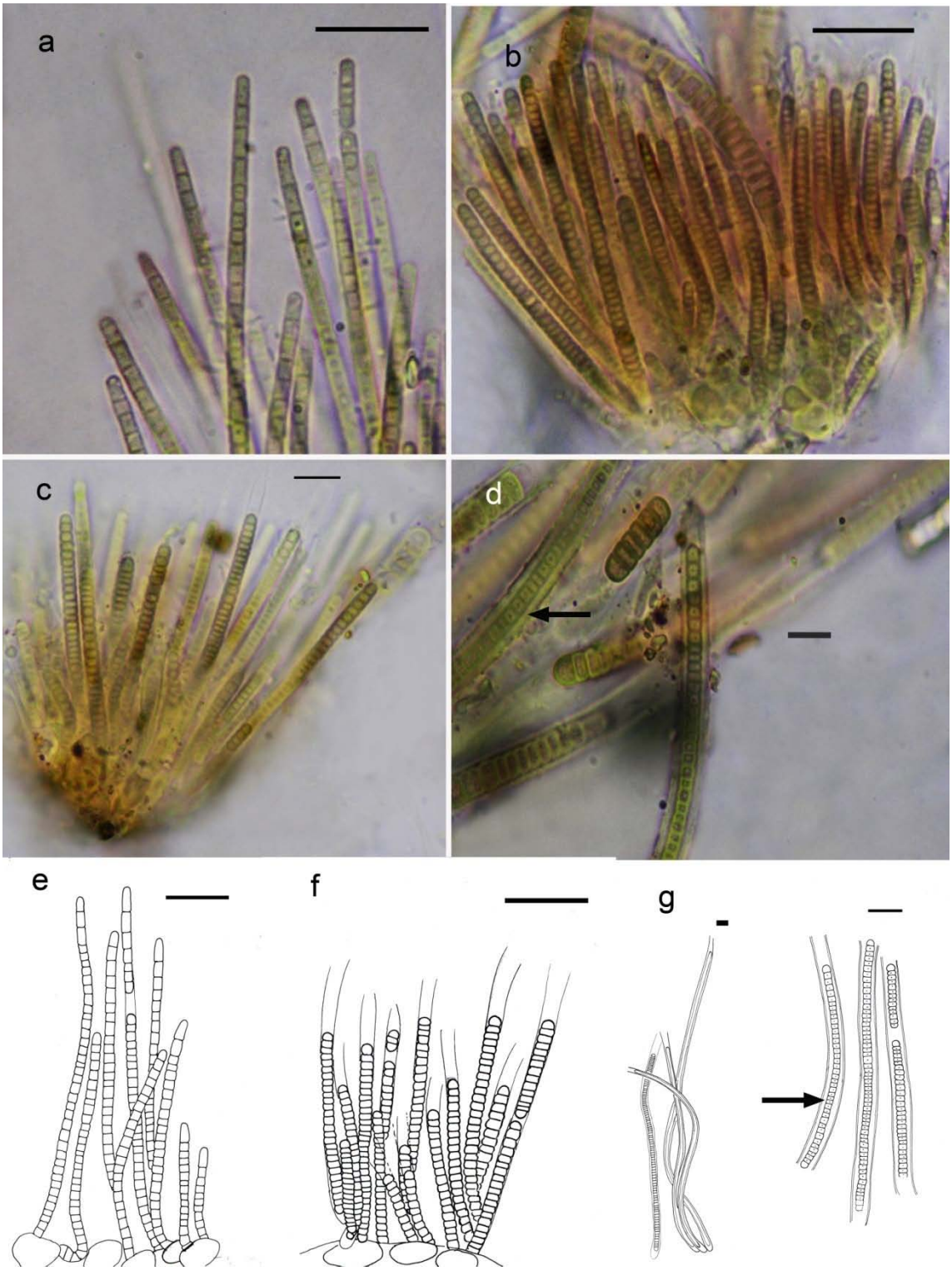

Figure 3.11: ***Heteroleibleinia*** cf. ***kossinskajae*****:** field specimens, **a, e,** trichome morphology with elongated apical cells. ***Heteroleibleinia*** cf. ***pusilla*****:** field specimens, **b,** dense aggregation of filaments appears reddish brown; **c**, **f,** short filaments with barrel-shaped cells. ***Heteroleibleinia*** cf. ***versicolor*****:** field specimens, **d, g,** filaments with distinctly widened sheath (arrow). Scale bars: 10 µm for a, b, e, f; 5 µm for c, d, g.

Homoeothrix gracilis Geitler 1927
Komárek and Anagnostidis (2005): p267, fig. 356 (p268)

Description: Field specimens form thin black crust. Filaments blue-green, heteropolar with one end attached to substratum, straight or slightly flexuous, aggregated densely (Fig. 3.12a). Trichomes 3.1-3.7 µm wide at base, 1.8-2.5 µm wide in centre, gradually tapering towards a terminal hyaline hair, distinctly constricted at cross walls. Cells almost isodiametric or shorter than wide, 0.6-2.5 µm long. Sheath firm, narrow, usually hyaline in the upper part, intensely golden-yellow at the base, occasionally this pattern is reversed (Fig. 3.12b, f).
Occurrence: Epilithic in unshaded fourth order stream.
Remarks: Specimens fit the description by Komárek and Anagnostidis (2005) with cell width on the lower end of the given range. Sheath characteristic is very prominent with an intense golden-brown pigment at the base. Basal vegetative cells were not visible.

Previous collections were from oligotrophic shallow waters, epilithic, forming thin brownish-green crusts; also on limestone substrata in streams and in littoral of small lakes and pools in the Austrian Alps (Komárek and Anagnostidis, 2005).

Homoeothrix cf. ***varians*** Geitler 1927
Komárek and Anagnostidis (2005): p265, fig. 353 (p266)

Description: Field specimens are frequent components of crusts as for *Chamaesiphon* cf. *subglobosus* (Fig. 3.10a), also occasionally epiphytic on the filamentous chlorophyte *Cladophora* as solitary filaments. Filaments brownish yellow, heteropolar with one end attach to the substratum, long, straight or slightly flexuous, aggregated densely into tufts (Fig. 3.12c). Trichomes 3.5-5.0 µm wide, constricted at cross walls, tapering to a hyaline hair of varying width and length (Fig. 3.12d). Cells almost isodiametric or shorter than wide, 1.0-3.0 µm long. Sheath firm, thin, hyaline.
Occurrence: Epilithic in unshaded second order stream.
Remarks: *Homoeothrix varians* (Komárek and Anagnostidis, 2005) has wider filaments (2.0-3.6 µm) than the specimen in this study.

Previous records from stones in cold, slow or rapidly flowing mountain streams and epiphytic on aquatic plants (Komárek and Anagnostidis, 2005).

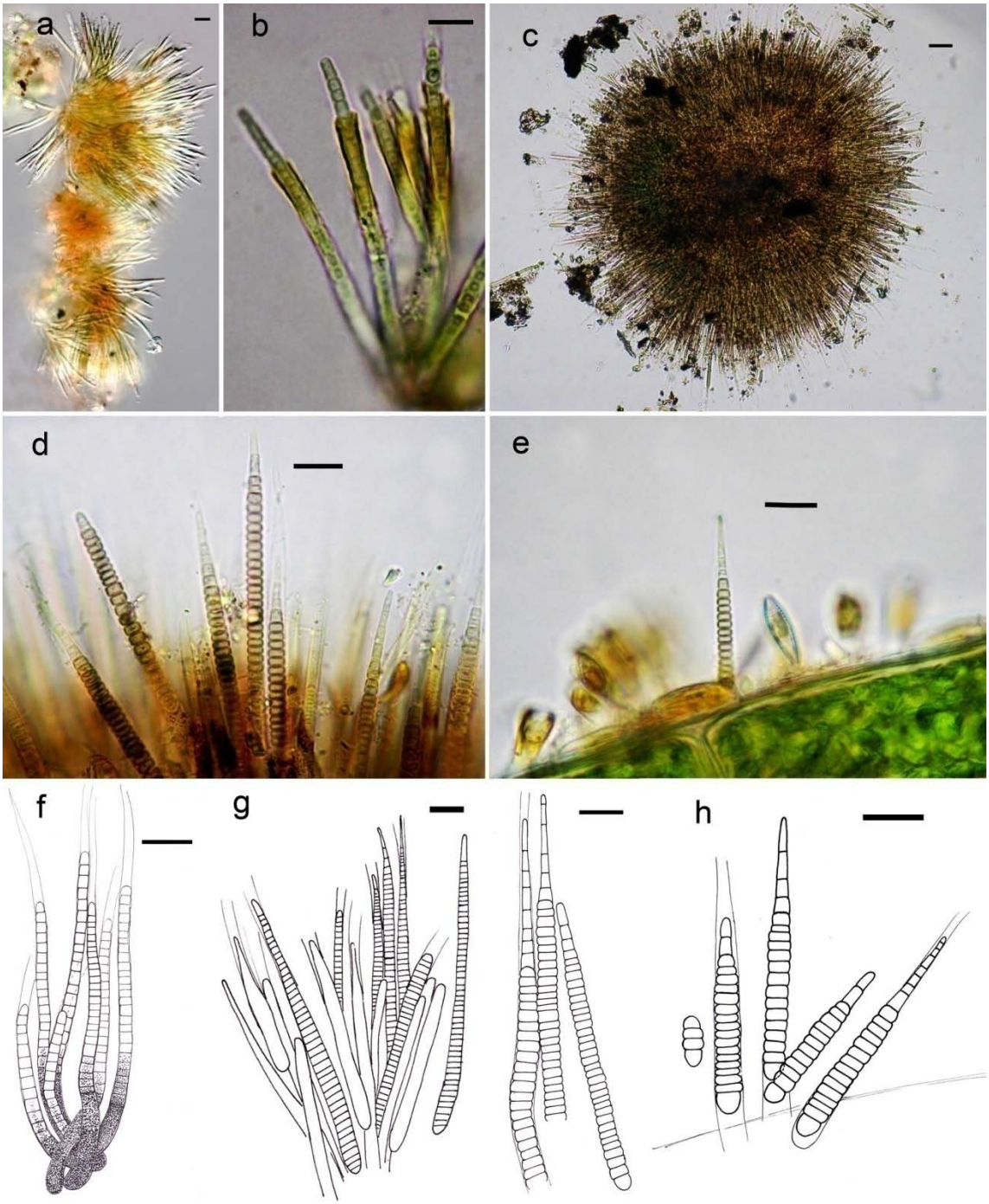

Figure 3.12: ***Homoeothrix gracilis*****:** field specimens, **a,** dense aggregation of filaments; **b,** trichome morphology with golden brown sheath; **f, highly** granulated golden-brown sheath at the base of the filament. ***Homoeothrix* cf. *varians*:** field specimens, **c,** colony morphology with densely aggregated filaments; **d,** trichome morphology with tapering apical cells; **e, h,** trichome morphology of the epiphytic form. **g,** aggregations formed with *Heteroleibleinia* morphospecies. Scale bars: 5 µm for b, d-h; 10 µm for a,c.

Leptolyngbya cf. ***bijugata*** (Skuja) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p206

Description: Filaments entangled amongst growths of *Phormidium* mats, bright blue-green, long, flexuous (Fig. 3.13a), with slow gliding motility. Trichomes 1.5-2.0 µm wide, constricted at cross walls, not or slightly attenuated at the ends. Cells isodiametric or longer than wide, 2.0-3.5 µm long, with a granule at each end (Fig. 3.13f). Apical cell conically rounded, without a calyptra. Sheath thin, firm, hyaline.

Colonies on agarised culture medium thin, bright blue-green spreading . Filament morphology similar to field material except trichomes slightly narrower width, (1.0) 1.5-1.8 µm.

Occurrence: A common associate amongst mats of *Phormidium* in unshaded fourth order stream.

Remarks: *Leptolyngbya bijugata* is placed in subgenus *Protolyngbya* based on cells being distinctly longer than wide and the absence of necridic cells (Komárek and Anagnostidis 2005). Trichome morphology differs from the description by Komárek and Anagnostidis (2005) only in its occasional slight terminal attenuation.

Previous records were from epipelon associated with mud in stagnant water of ponds and lakes in Russia, Ukraine and central Asia (Komárek and Anagnostidis 2005).

Leptolyngbya foveolarum (Rabenhorst ex Gomont) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p 188, fig. 229 (p191)

Descriptions: Field specimens entangled within *Rivularia* colonies (Fig. 3.13b, c). Filaments pale blue-green, isopolar, long, straight or flexuous. Trichomes 1.5-2.0 µm wide, distinctly constricted at cross walls, not attenuated or slightly attenuated and bent at the ends (Fig. 3.13g), with slow gliding motility. Cells isodiametric or shorter than wide, 1.0-2.0 µm long. Apical cell obtuse rounded or conically rounded, without a calyptra. Sheath thin, firm, hyaline.

Occurrence: Commonly noted within *Rivularia* colonies in unshaded fourth order stream.

Remarks: Specimens comply with Komárek and Anagnostidis (2005) concept of the species apart from occasional attenuation of trichomes.

Leptolyngbya foveolarum is widely distributed with records from submersed stones and rocks to moist soils, ditches with polluted waters, greenhouses and at the margins of thermal and mineral springs (Komárek and Anagnostidis 2005).

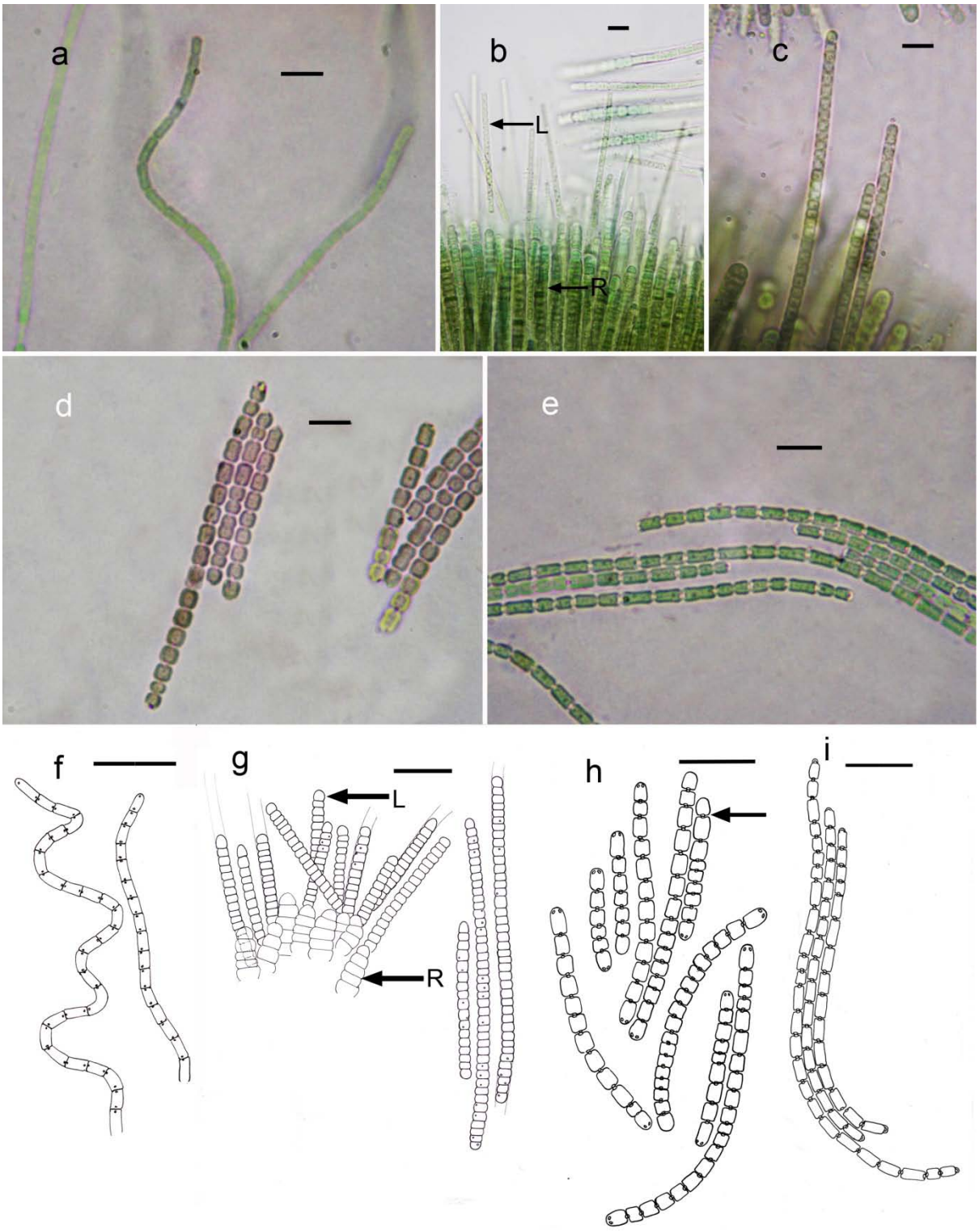

Figure 3.13: ***Leptolyngbya*** cf**.** ***bijugata*****:** field specimens, **a**, narrow trichomes constricted at cross-walls; **f,** trichomes with cells longer than wide, granulated at cross walls. ***Leptolyngbya foveolarum*****:** field specimens, **b g,** filaments (arrow L) released from a colony of *Rivularia* (arrow R); **c,** trichome with barrel-shaped cells and rounded apex. ***Pseudanabaena*** cf**.** ***amphigranulata*****:** cultures, **d,** short isopolar trichomes with isodiametric cells, constricted at cross wall; **h,** cells with 1 or 2 aerotopes at cross walls (arrow). ***Pseudanabaena*** cf. ***galeata*****:** cultures, **e,** trichome morphology with cells longer than wide; **i,** crescent-shaped aerotopes at apex of terminal cell. Scale bars: 5 µm for a-e; 10 µm for f-i.

Pseudanabaena amphigranulata (Van Goor) Anagnostidis 2001
Komárek and Anagnostidis (2005): p86, fig. 65 (p87)

Description: Colonies on agarised culture medium purplish. Trichomes blue-green to purplish, isopolar, short, bent, rarely straight, 1.5-2.0 µm wide, usually with few cells (6-25), distinctly constricted at cross walls, not attenuated at the ends, motile with slow gliding movements (Fig. 3.13d). Cells isodiametric or longer than wide, slightly barrel shaped, 1.5-3.0 µm long, with 1 or 2 aerotopes at cross walls (Fig. 3.13h). Apical cell obtuse rounded with two apical aerotopes. Sheath absent.

Remarks: Specimens fit the description of *P. amphigranulata* (Komárek and Anagnostidis, 2005). *Pseudanabaena amphigranulata* was recorded from cultures inoculated with mats of *Oscillatoria limosa*. The specimen was originally described with *O. limosa*, *Phormidium chalybeum* and purple sulphur bacteria in shallow eutrophic lakes with muddy sediments (Komárek and Anagnostidis, 2005).

Pseudanabaena galeata Böcher 1949
Komárek and Anagnostidis (2005): p88, fig. 67 (p87)

Description: Colonies on agarised culture medium bright blue-green mats. Trichomes pale blue-green, isopolar, short, flexuous, rarely straight, 1.0-1.5 µm wide, with 15-40 cells, constricted at cross walls, not attenuated , with slow gliding motility (Fig. 3.13e). Cells longer than wide, cylindrical, 3.0-3.5 µm long, with an aerotope at each cross wall. Apical cell conically rounded with a characteristically crescent shaped, apical aerotope (Fig. 3.13i). Sheath absent.

Remarks: Specimens were recorded only from cultures inoculated with mats of *Phormidium* cf. *bekesiense*. Trichome width is within the lower end of the range given by Komárek and Anagnostidis (2005). They state that the distinct aerotope at the apex of the terminal cell is characteristic of the species.

Pseudanabaena galeata has been reported from mud surfaces and as an epiphyte on aquatic plants. It is widely distributed (Komárek and Anagnostidis 2005).

Family **Phormidiaceae**

Phormidiochaete sp.
Komárek and Anagnostidis (2005): p554

Description: Field specimens form thin, black, epilithic crusts. Filaments blue-green, heteropolar with one end attached to substratum, long, flexuous, densely aggregated to form tufts (Fig. 3.14i). Trichomes 5.0-6.3 µm wide distally and tapered towards apex, 6.3-7.5 µm wide towards the base,

constricted at cross walls. Cells shorter than wide towards the apex, 1.3-5.0 µm long; isodiametric to longer than wide toward the base, 5.0-8.8 µm long. Sheath firm, thin, hyaline.

Occurrence: Epilithic in unshaded fourth order stream.

Remarks: The present specimens are assigned to *Phormidiochaete* based on trichome width which is larger than 4.0 µm and distinctly longer than wide cells especially towards the base. Only three morphospecies of *Phormidiochaete* are described by Komárek and Anagnostidis (2005). The present specimens cannot be assigned to any of these because of the distinctly longer cells towards the base.

Phormidium autumnale *(*Agardh) Trevisan ex Gomont 1892
Komárek and Anagnostidis (2005): p473, fig. 707 (p474). McGregor (2007): p52, fig. 9B (p53). Whitton (2011): p101, fig. 22C (p102)

Description: Mats in the field are olive green (Fig. 3.14a) or purplish-red (Fig. 3.14b), thick, leathery, strongly coherent and extensive. Trichomes blue-green, long, straight or slightly flexuous, usually entangled, 6.0-7.0 µm wide, not constricted at cross walls, abruptly attenuated at the ends, motile with strong oscillation and anticlockwise rotation (Fig. 3.14c, d). Cells isodiametric or shorter than wide, 2.0-5.0 µm long (Fig. 3.14f, g). Apical cell rounded to conical, often capitate with calyptra. Sheath absent.

Mats on agarised culture medium olive green, patterned with swirls. Trichome morphology similar to field specimens except for the olive green colour and granulation (Fig. 3.14e, h).

Occurrence: Epilithic in unshaded fourth order stream. Extensive mats in runs and riffle.

Remarks: Differences observed in mat and trichome pigmentation is likely to be due to light intensity and quality (Whitton, 2011).

Previous records as epilithic in streams, rivers, tanks and waterfalls. Records from moist soils, walls, tree-bark, thermal springs and marine coastal rocks need confirmation (Komárek and Anagnostidis, 2005).

Phormidium *cf.* ***bekesiense*** (I.Kiss) K. Kiss in Anagnostidis 2001
Komárek and Anagnostidis (2005): p465, fig. 686 (p467)

Description: Mats in the field on silt, bright blue-green, soft, slimy, loosely coherent, with an undulating surface pattern (Fig. 3.15b). Trichomes bright blue-green, long, straight or slightly flexuous (Fig. 3.15c), (7.5) 9.0-10.0 µm wide, not constricted at the ungranulated cross walls, intensely motile, sometimes quite jerky, with strong oscillation of the tip and clockwise rotation; mature terminal regions are slightly attenuated and distinctly yellowish to very pale blue-green. Cells isodiametric or shorter than wide, 6.0-10.0 µm long (Fig. 3.15d). Apical cell capitate, obtuse rounded (Fig 3.15 e, g). Sheath absent.

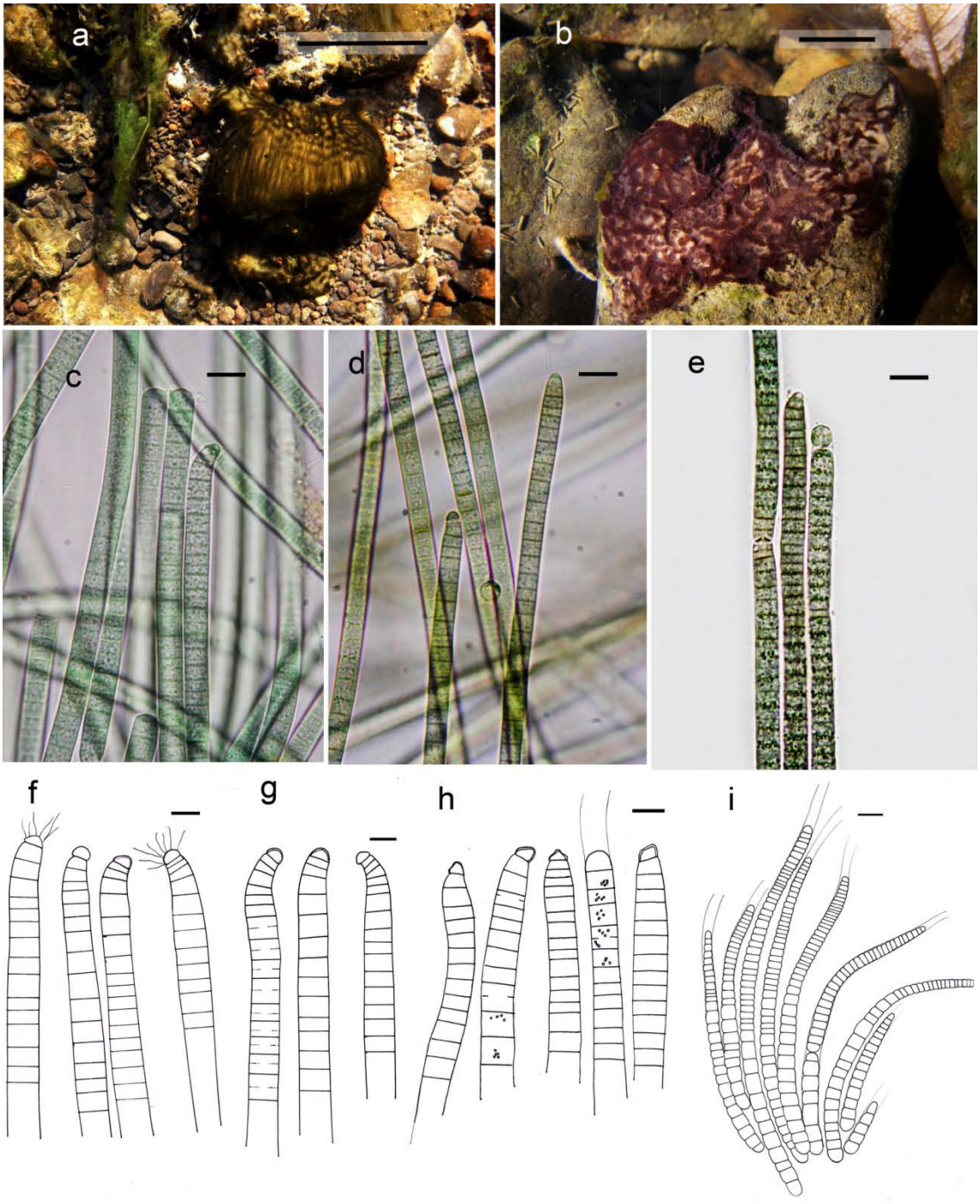

Figure 3.14: ***Phormidium autumnale*****:** field specimens, **a,** olive-green mats*;* **b,** purple mats; , **c**,**d**, trichome morphology with isodiametric to shorter than wide cells; **f**, **g,** variation in apical cell morphology, mature trichomes with calyptra; cultures, **e**, trichomes with densely granulated cells; **h,** apical cell morphology with calyptra in mature trichomes. ***Phormidiochaete*** sp.: field specimens, **i**, trichome morphology showing variation in cell length, distinctly longer cells occur towards the base. Scale bars: 5 µm for c-h; 10 µm for i, 5 cm for a-b.

Mats on agarised culture medium bright blue-green, thin, without patterning. Trichomes differ from field material only in narrower trichome width (7.0-8.0 µm) and occasional presence of sheath (Fig. 3.15f, h).

Occurrence: Epipelic in pools, very slow runs and at stream margins, outside major flow in partly shaded third and fourth order streams. The mats co-occur with *P.* cf. *formosum* (Fig. 3.15a, b).

Remarks: *Phormidium bekesiense* has narrower trichomes (8.0-9.0 µm wide) than Kaituna field specimens but wider trichomes than cultured specimens. The present specimens could not be confidently assigned to the species.

Previous collection was from periodically drying freshwater localities and has been found repeatedly in southern Hungary (Komárek and Anagnostidis, 2005).

Phormidium chalybeum (Mertens ex Gomont) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p422, fig. 604 (p423). McGregor (2007): p55, fig. 9D (p53)

Description: Mats in field dark blue-green, soft, fragile, loosely coherent, with long strands trailing in the flow (Fig. 3.16a). Trichomes blue-green, long, straight to slightly curved (Fig. 3.16b), (7.5) 8.0- 10.00 µm wide, clearly constricted at cross walls, slightly attenuated at the ends, motile with slow jerky gliding. Cells isodiametric or shorter than wide, (3.8) 5.0-10.0 µm long. Apical cell conically to broadly rounded, obtuse, without a calyptra (Figs. 3.16c-g). Cell content finely granulated (Fig. 3.16h) with occasional prominent large granule. Sheath absent.

Occurence: Epilithic in unshaded fourth order stream.

Remarks: Specimens have trichome width at the larger end of the range [(6.0) 7.0-8.5 (13.0) µm wide] described by Komárek and Anagnostidis (2005).

Widely distributed on stones, mud and wood in streams, springs and stagnant waters (Komárek and Anagnostidis, 2005).

Phormidium inundatum Kützing ex Gomont 1892
Komárek and Anagnostidis (2005): p430, fig. 618 (p429)

Description: Mats in field dark blue-green, thin and coherent (Fig. 3.17a). Filaments bright blue-green, long, straight or flexuous, usually loosely entangled. Trichomes 4.0-5.0 µm wide, not constricted at cross walls, slightly attenuated at the ends, with slow gliding motility, (Fig. 3.17b). Cells almost isodiametric or slightly shorter or longer than wide, (3.0) 5.0-7.0 µm long, granulated at cross-walls. Apical cell slightly narrowed, distinctly conical or rounded conical, without a calyptra (Figs. 3.17c, e). Sheath thin, hyaline and always present.

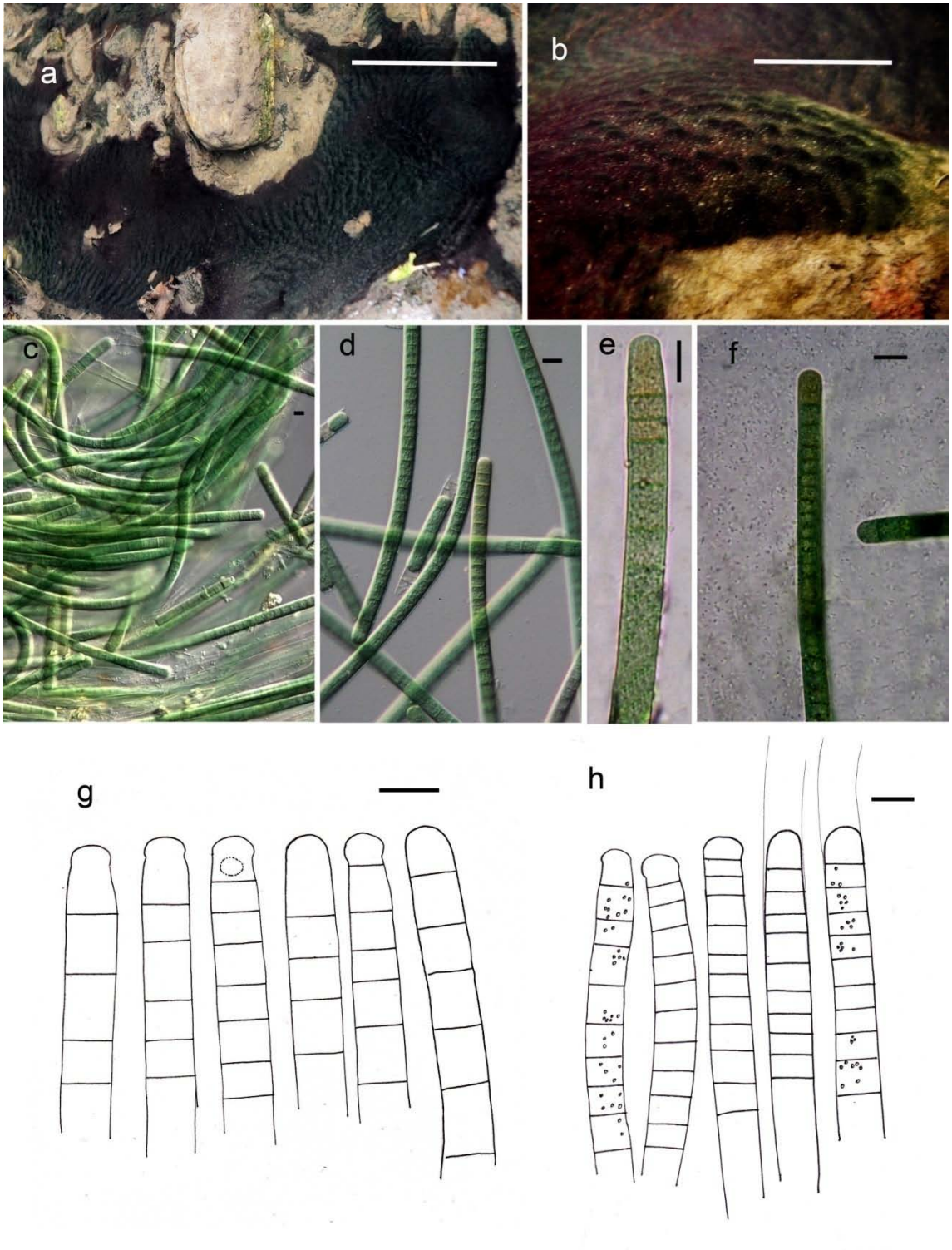

Figure 3.15: ***Phormidium*** cf. ***bekesiense*****:** field specimens, **a,** dark blue-green mats with *Oscillatoria* cf. *simplicissima* purplish-black mats around the edge; **b,** the texture of mats co-occuring with *O.* cf. *simplicissima*; **c,** long, flexuous and entangled trichomes; **d,** typical trichomes with cells isodiametric or shorter than wide; **e**, slightly tapered mature apical cell; **g,** apical cell variation; cultures, **f**, trichome with narrower width than field specimens; **h,** apical cell variation. Scale bars: 10 µm for c-h; 30 cm for a; 5 cm for b.

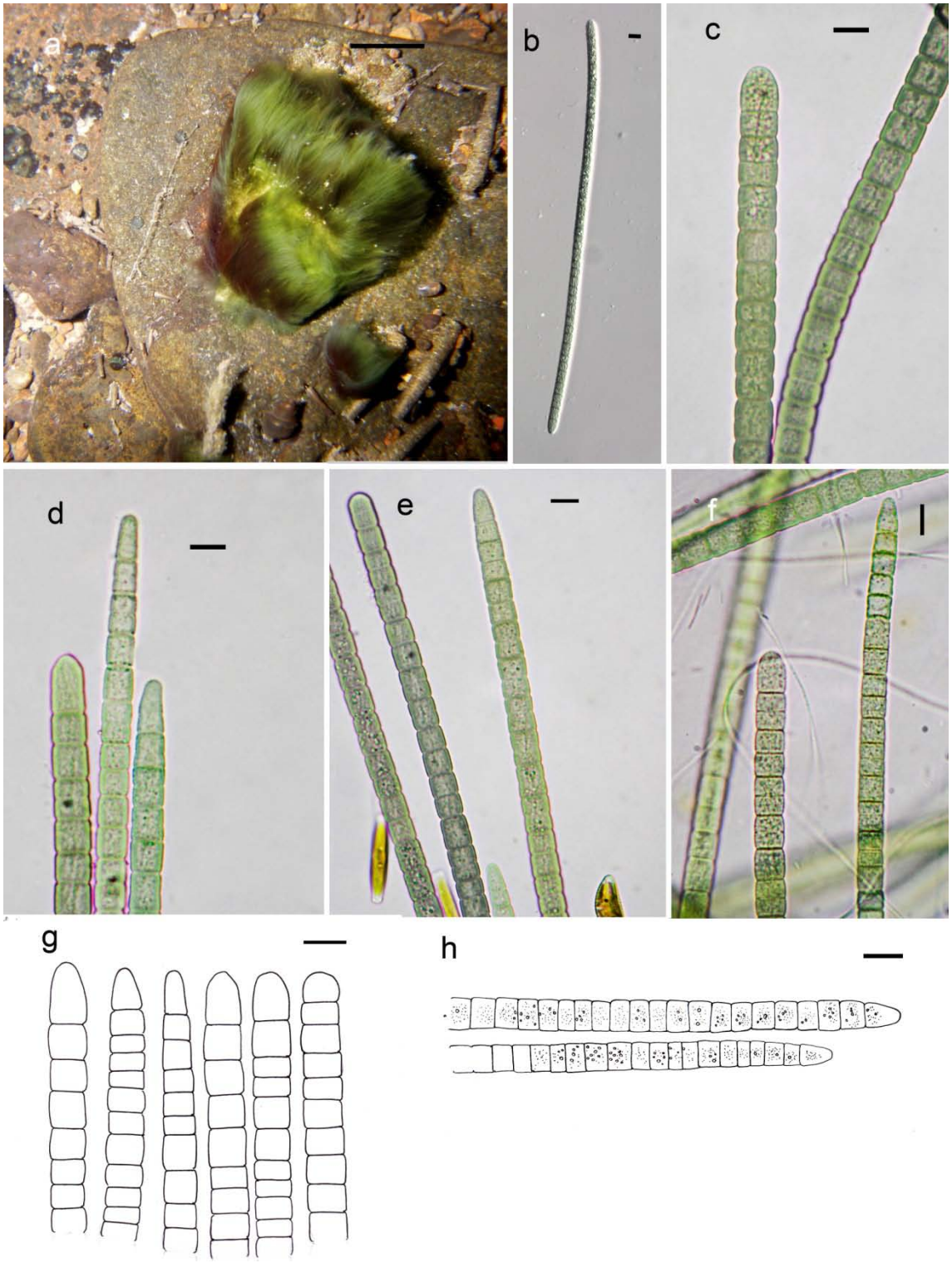

Figure 3.16: ***Phormidium chalybeum***: field specimens, **a**, blue-green mats with strands trailing in the flow; **b,** trichome with mature apices at each end**; c-g**, variation in apical cell morphology; **h,** Cells with large granules as occasionally noted. Scale bars: 10 µm for b-h; 2 cm for a.

Mats on agarised culture medium bright blue-green, thin. Filament morphology identical to field specimens (Fig. 3.17d, f).

Occurence: Epilithic in partly shaded third order stream, only at site 78.

Remarks: Closely fits the description given by Komárek and Anagnostidis (2005).

Previously recorded from oligotrophic and mesotrophic flowing and stagnant waters on submerged vegetation (Komárek and Anagnostidis, 2005). The present collection extends its habitat range to rock surfaces.

Phormidium cf. ***irriguum*** (Kützing x Gomont) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p481, fig. 719 (p482); McGregor (2007): p56, fig. 10B (p54)

Description: Mats in the field dark-brown, slimy, loosely wrapping around, and sometimes completely obscuring, stems and leaves of macrophytes (Fig. 3.18a). Trichomes olive-green, isopolar, long, flexuous, rarely straight, 11.0-12.0 µm wide, not constricted at cross walls, attenuated towards the apex (Fig. 3.18b), with slow gliding motility. Cells shorter than wide to almost isodiametric, 2.5-8.0 µm long. Apical cell obtuse rounded or conically rounded with a distinct calyptra. Sheath absent (Fig. 3.18h).

Mats on agarised culture medium brown, thin, slimy. Trichome morphology identical to field specimens.

Occurence: Epiphytic in unshaded fourth order stream at site 47.

Remarks: *Phormidium irriguum* has narrower trichomes (6.0-11.2 µm) which are purple-greyish in colour. Cell length is generally greater (4.0-11.0 µm) than in the present specimens.

Previous collections were from stagnant and flowing waters, among other algae and also on moist rocks, logs and other debris in coastal streams and rivers (Komárek and Anagnostidis, 2005; McGregor, 2007).

Phormidium cf. ***subfuscum*** (Kützing ex Gomont) Anagnostidis et Komárek 1988
Komárek and Anagnostidis (2005): p481, fig. 721 (p482)

Description: Filaments brown, long, straight, solitary (Fig. 3.18d, e). Trichomes 10.0-11.0 (12.5) µm wide, not constricted at cross walls, slightly attenuated at ends, with slow, gliding motility. Cells almost isodiametric to shorter than wide, 2.0-5.0 µm long. Apical cell conical or slightly rounded with calyptra (Fig. 3.18i). Sheath occasionally present, thin and hyaline.

Mats on agarised culture medium reddish-brown, thin. Trichomes 12.0 µm wide (Fig. 3.18f), occasionally with slight constrictions at cross walls. Sheath often present.

Occurence: A minor component within mats of *P. inundatum* (Fig. 3.18c) in partly shaded third order stream at site 78.

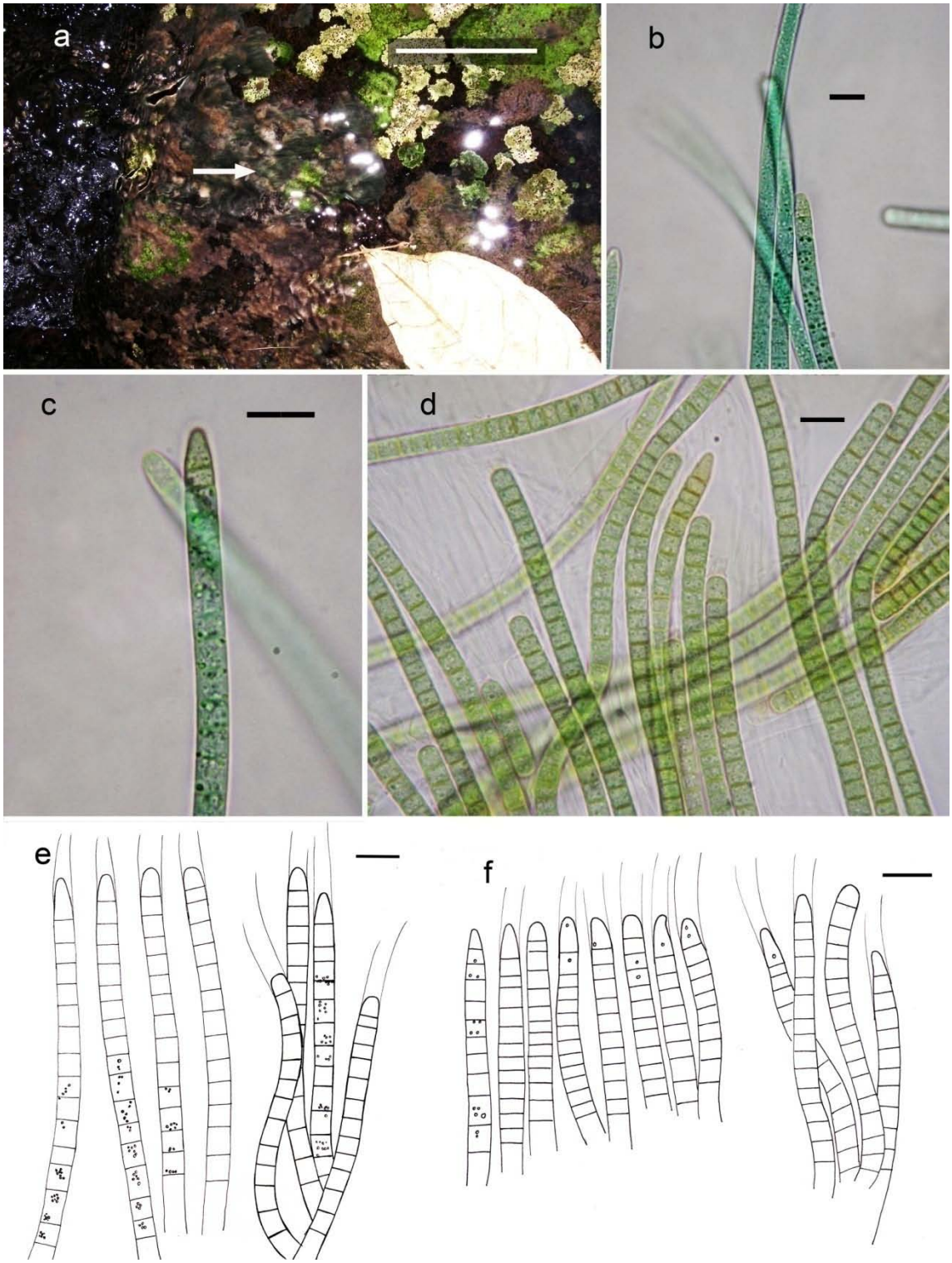

Figure 3.17: ***Phormidium inundatum*****:** field specimens, **a,** dark blue-green thin mats; **b,** trichome bright blue-green, straight to slightly flexuous; **c,** mature conical apical cell; **e,** variation in apical cell shape; cultures, **d, f** variation in trichome morphology and apical cell shape. Scale bars: 10 µm for b-h; 2 cm for a.

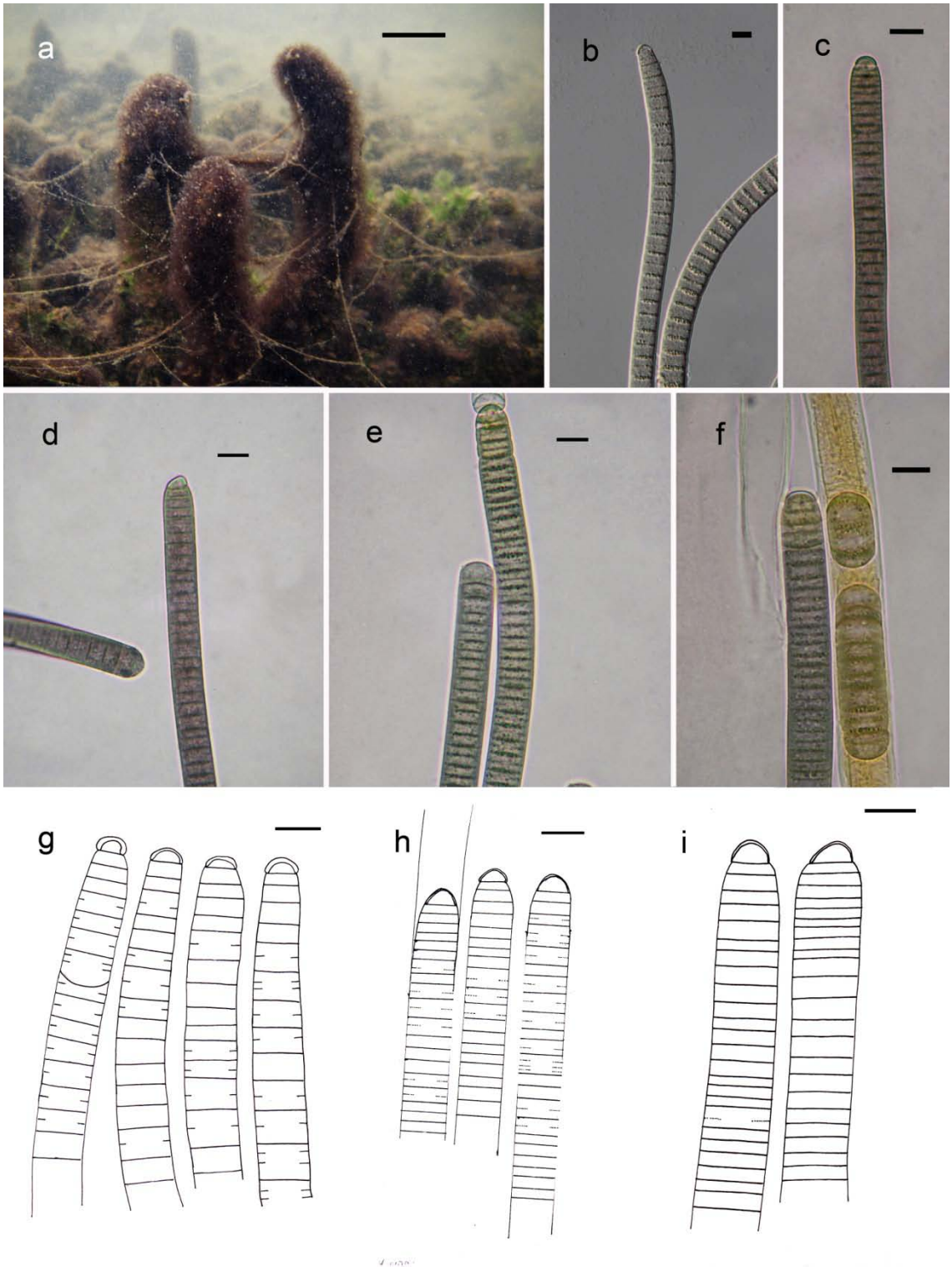

Figure 3.18: ***Phormidium*** cf. ***irriguum*****:** field specimens, **a,** brown mats epiphytic on aquatic angiosperms, where mats are thick and extensive they completely cover the angiosperms; **b,** trichome short to almost isodiamtric cells, not constricted at cross wall; **g**, mature trichomes with apical cells with distinct calyptra**.** ***Phormidium*** cf. ***subfuscum*****:** field specimens**, c, d, h,** trichome with short cells, not constricted a cross wall, apical cell calyptrate; cultures**, e, f, i,** trichome and apical cell morphology similar to field specimens. Scale bars: 10 µm for b-j; 2 cm for a.

Remarks: Unlike the present specimens, *P. subfuscum* has a diffluent, lamellated sheath and granulation at cross walls.

Phormidium subfuscum recorded particularly in limestone regions, on rocky or woody substrata in rivers and streams, less frequently in stagnant waters of water tanks and ponds (Komárek and Anagnostidis, 2005).

Phormidium uncinatum Gomont ex Gomont 1892
Komárek and Anagnostidis (2005): p481, fig.719 (p482); McGregor (2007): p60, fig. 11A (p59)

Description: Mats in the field reddish-brown (Fig. 3.19a), yellowish-brown (Fig. 3.19b) or dark-brown (Fig. 3.19c). Trichomes olive-green or pale blue-green, long, straight or slightly bent, (7.0) 8.0-9.0 µm wide, not constricted at cross walls, abruptly attenuated at the ends (Figs. 3.19e, g, h), vigorously motile with anticlockwise rotation. Cells shorter than wide, 2.0-6.0 µm long. Apical cells with obtuse or conically rounded calyptras (Fig. 3.19g). Sheath absent.

Mats on agarised culture medium dark-brown, thin, slimy. Trichome morphology is identical to field material (Figs. 3.19f, g).

Occurrence: Epilithic in unshaded fouth order stream. Mats are extensive in runs and riffles.

Remarks: It is difficult to distinguish *P. uncinatum* from *P. autumnale*. Komárek and Anagnostidis (2005) separated the two morphospecies based on trichome width and cell shape: *P. uncinatum* having generally wider trichomes, (4.0) 5.5-9.0 (9.5) µm and shorter cells, *P. autumnale* having narrower trichomes (≤7.0 µm) and cells isodiametric or longer than wide. Whitton (2011) merged the two morphospecies under *P. autumnale* based on the overlap between their trichome width ranges (4-7 µm cf. 5.5-9.0 µm). Geitler (1932) regarded them as separate entities but expressed doubt. Here the trichome morphology suggests a closer relationship to *P. uncinatum*.

Previous collections from rocks, stones, woods, other substrata from flowing and stagnant waters (Komárek and Anagnostidis, 2005) and amongst filamentous algae and aquatic macrophytes (McGregor, 2007). Widely distributed.

Family **Oscillatoriaceae**

Homoeothrix juliana (Lemmermann) Lemmermann 1907
Komárek and Anagnostidis (2005): p652, fig. 1010 (p654). McGregor (2007): p71, fig. 13A (p 69). Whitton (2011): p84, pl. 17A (p86)

Description: Crusts in field black, slimy, thin. Trichomes pale blue-green, heteropolar, flexuous, solitary or closely aggregated in parallel in small groups, 12.0-14.0 µm wide at base, 10.0-12.0 µm wide in the middle, gradually tapering towards an apical hyaline hair, distinctly constricted at cross

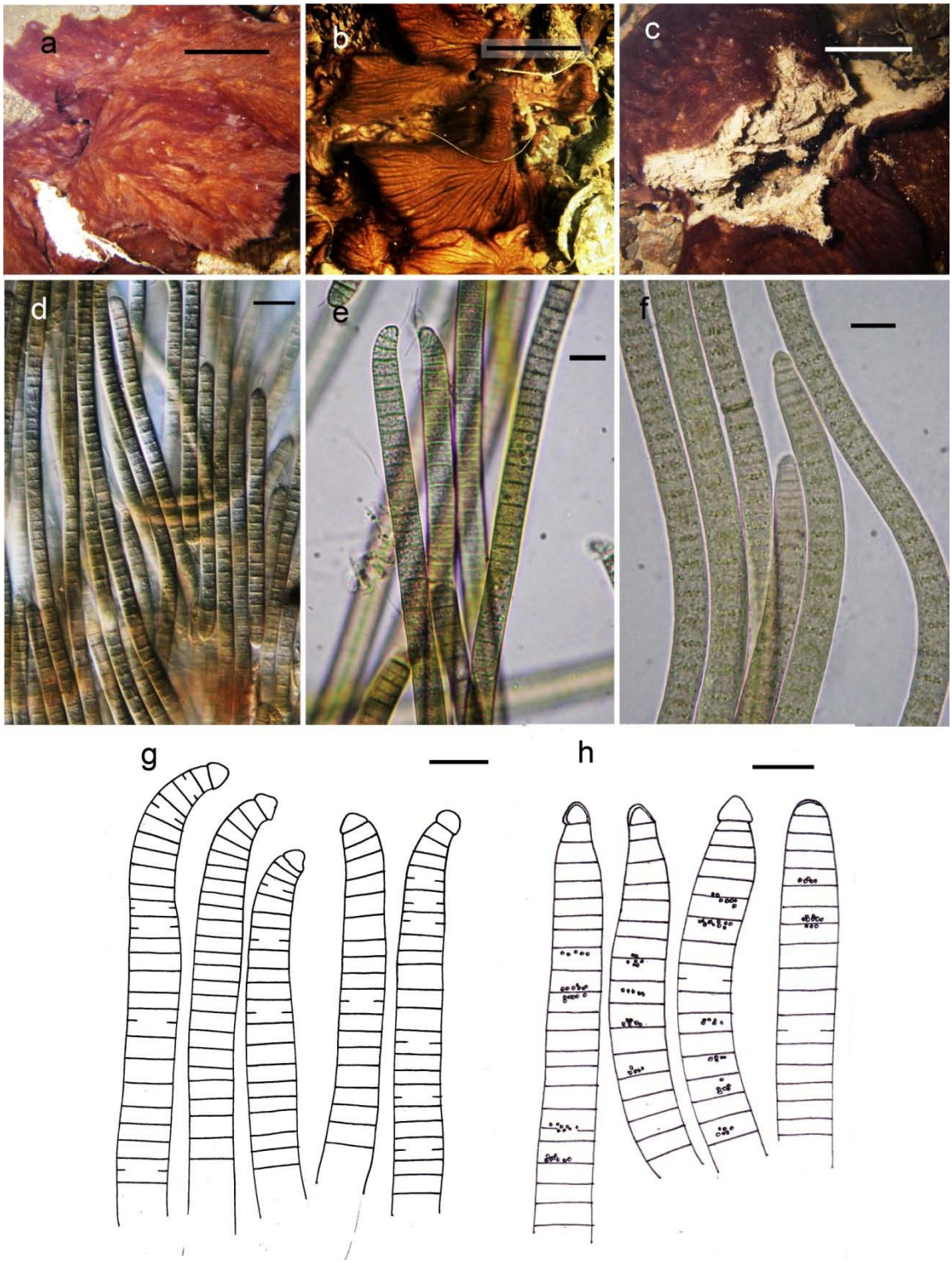

Figure 3.19: ***Phormidium*** cf. ***uncinatum*****:** field specimens, **a-c,** mats showing variation in colour from purplish-red to yellowish-brown to dark purple*;* **d,** trichomes densely packed within the mats; **e, g,** variation in trichome and apical cell morphology; cultures, **f, h,** variation in trichome and apical cell morphology,trichome granulated. Scale bars: 10 µm for d-h; 2 cm for a-c.

walls (Fig. 3.20d). Cells distinctly shorter than wide, 2.0-8.0 µm long., Basal cell broadly rounded. Sheath firm, thin, hyaline.

Occurence: Rarely observed, epilithic in unshaded fourth order stream.

Remarks: Specimens conform closely to *H. juliana* except for the formation of hyaline hairs in Kaituna specimens.

Previous records from flowing waters on stones, calcareous substrata and shells, often forming crusts; rarely among other algae (Komárek and Anagnostidis, 2005). McGregor (2007) reported its occurrence in alkaline coastal streams on stones and aquatic macrophytes.

Oscillatoria curviceps Agardh ex Gomont 1892
Komárek and Anagnostidis (2005): p589, fig. 879 (p588). McGregor (2007): p76, fig. 14I (p70)

Description: Trichomes bright blue-green, long, straight or slightly flexuous, 10.0-12.0 µm wide, not constricted at cross walls, occasionally with slight attenuation towards apex, slow gliding motility (Fig. 3.20a). Cells distinctly shorter than wide, 1.2-3.8 µm long (Fig. 3.20e). Apical cell obtuse rounded, lacking a calyptra, occasionally slightly bent. Sheath occasionally present.

Occurence: Commonly found at low abundance in mats of *Phormidium* morphospecies in unshaded fourth order stream.

Remarks: Komárek and Anagnostidis (2005) reported *O. curviceps* as usually solitary filaments among other periphytic cyanobacteria in stagnant and flowing waters.

Oscillatoria limosa Agardh ex Gomont 1892
Komárek and Anagnostidis (2005): p593, fig. 886 (p595); McGregor (2007): p76, fig. 15B (p77), Whitton (2011): p99, pl. 20G, H (p96)

Description: Mats in field blackish-brown, soft, slimy, loosely coherent, prostrate or entangled and wrapped around aquatic angiosperms. Trichomes olive green to slightly brownish, long, straight to slightly flexuous, 10.00-13.8 µm wide, not constricted at cross walls, occasionally slightly attenuated towards the apex, with slow motility (Fig. 3.20c). Cells distinctly shorter than wide, 2.5-5.0 µm long, with granules along cross-walls (Fig. 3.20f). Apical cell obtuse rounded with calyptra. Sheath absent.

Occurrence: Epiphytic on macrophytes and epipelic on silt in unshaded fourth order stream at sites 1-17 where it flows through the orchard.

Remarks: Trichome width range is at the lower end of that in Komárek and Anagnostidis (2005) but matches the range recorded by McGregor (2007).

Previous records from sandy and muddy sediments in stagnant or slow flowing freshwaters (Komárek and Anagnostidis 2005).

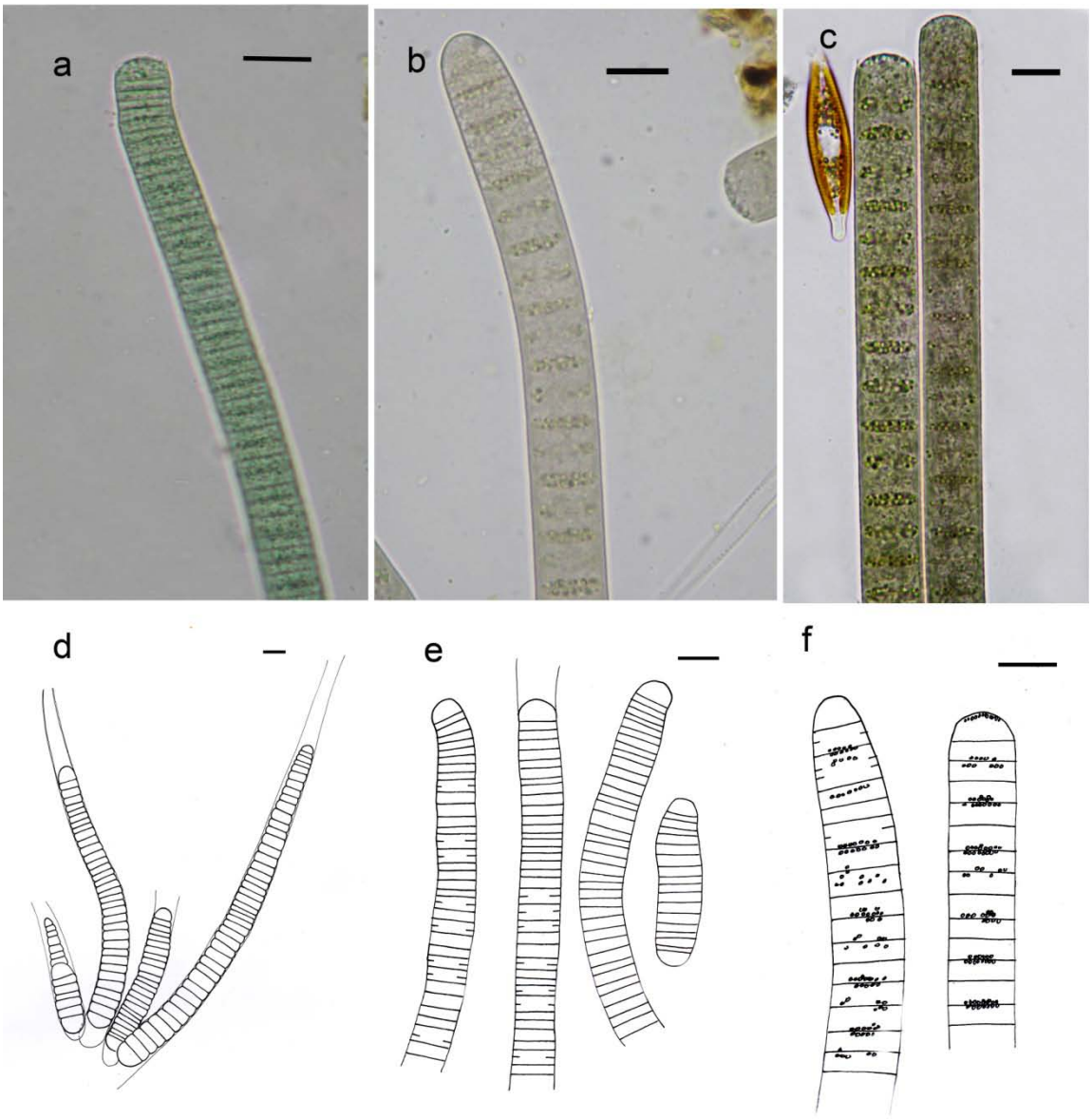

Figure 3.20: ***Oscillatoria curviceps*****:** field specimens, **a,** solitary trichome pigmented bright blue-green; **e**, trichome morphology showing distinctly short cells. ***Oscillatoria limosa*****:** field specimens, **b, c, f,** trichomes with granules along cross-walls. ***Homoeothrix juliana*****:** field specimens, **d,** heteropolar filaments with distinctly short cells. Scale bars: 10 µm for a-f.

Oscillatoria cf. ***simplicissima*** Gomont 1892
Komárek and Anagnostidis (2005): p586, fig. 876 (p585)

Description: Mats in field purplish-black, soft, slimy, loosely coherent (Fig. 3.21a). Trichomes dark olive-green to slightly brownish, long, straight to flexuous, irregularly entangled forming tight bundles (Fig. 3.21b), broad, 9.0-10.0 µm wide, not constricted at cross walls, slightly attenuated and bent at ends, intensely motile with oscillation and clockwise rotation. Cells isodiametric to half as long as wide, (2.5) 3.8-6.0 µm long (Fig. 3.21c). Apical cell slightly narrowed or conically rounded, without a calyptra (Figs. 3.21d, g). Sheath absent.

Mats on agarised culture medium olive-green, soft, slimy, loosely coherent, patterned with swirls. Trichomes identical to field material (Figs. 3.21e, f, h).

Occurence: Epipelic on silt in partly shaded pools and slow runs of third order streams.

Remarks: *Oscillatoria simplicissima* has narrower trichome width (8.0-9.0 µm wide) (Komárek and Anagnostidis, 2005).

Previous records from stagnant and unpolluted flowing waters from temperate and tropical regions (Komárek and Anagnostidis, 2005).

Family **Nostocaceae**

Anabaena cf. ***inaequalis*** (Kützing) Bornet et Flahault 1886
Whitton (2011): p122, pl. 25D (p123). Geitler (1932): p896, fig. 578 (p897). Desikachary (1959): p413

Description: Mats in the field blue-green, soft, slimy, loosely coherent, prostrate (Fig. 3.22a). Trichomes bright blue-green, long, straight to slightly flexuous, 4.5-6.3 µm wide, distinctly constricted at cross walls (Fig. 3.22b). Cells barrel-shaped, short, 2.5-5.0 µm long. Apical cell broadly rounded to very slightly conically rounded. Heterocytes 6.0-7.5 µm wide, 8.5-12.0 µm long. Akinetes, single or many in a row, cylindrical with smooth outer wall, yellowish, formed distant from heterocytes, 7.5-8.5 µm wide, 20.0-30.0 µm long (Fig. 3.22e). Sheath absent.

Occurrence: Epipelic on silt in unshaded fourth order stream at site 24.

Remarks: Specimens have larger heterocytes and akinetes than *A. inaequalis* (6.0-10.0 x 5.5-6.5 µm and 14-20 x 6.0-8.0 µm respectively; Whitton, 2011; Geitler, 1932). Akinete dimensions fall within the size range for *A. oscillarioides* (20.0-40.0 x 8.0-10.0 µm) (Whitton, 2011; Desikachary, 1959). In this species, akinetes showed paraheterocytic development.

Anabaena inaequalis is known from standing freshwater, attached or free- floating (Smith and Lester, 2007; Whitton, 2011) and from brackish ponds and moist soils (Desikachary, 1959).

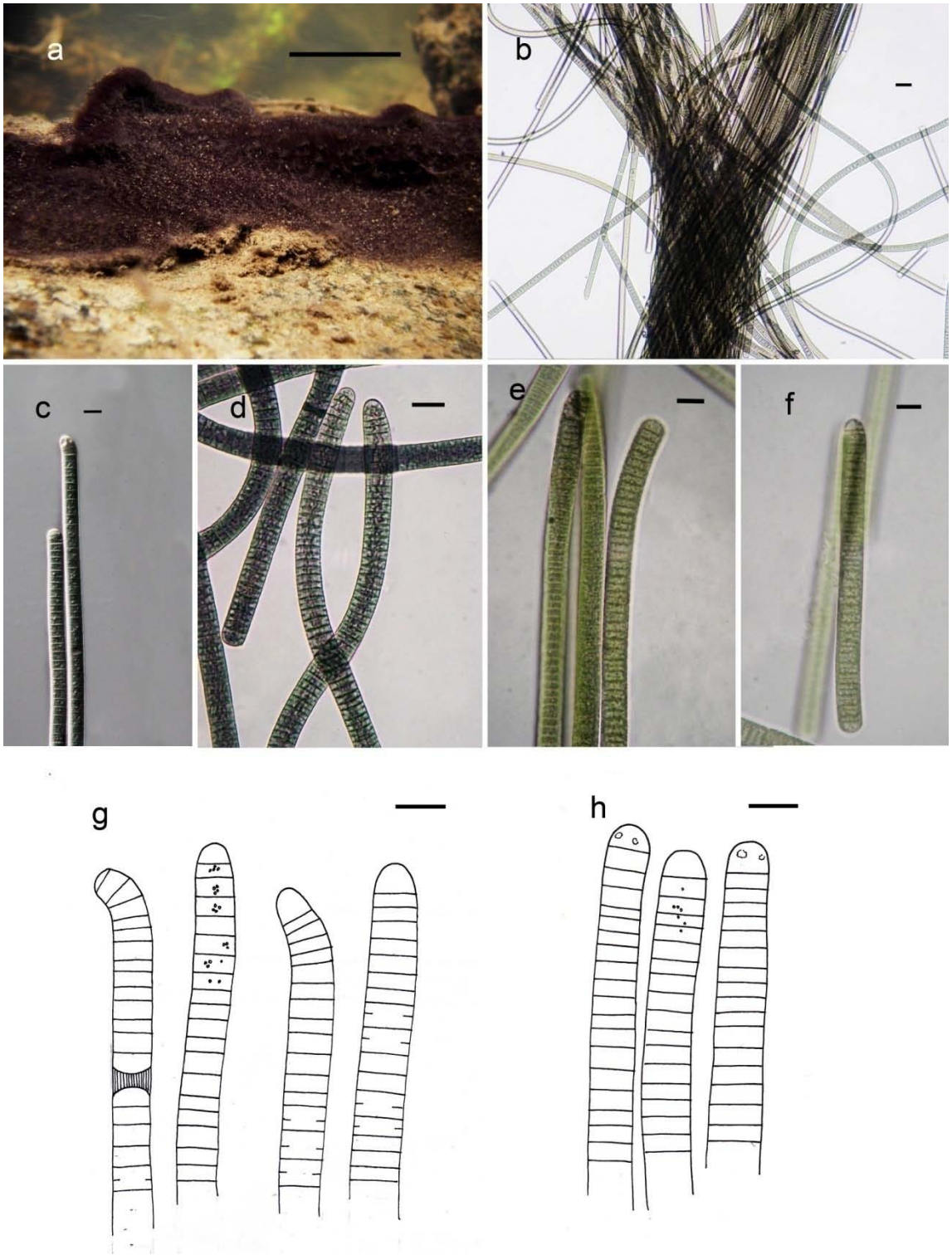

Figure 3.21: ***Oscillatoria*** cf. ***simplicissima*****:** field specimens, **a,** dark purple mats*;* **b,** entangled filaments**; c, d, g**, trichomes showing variation in terminal regions and apical cells; cultures, **e,f, h,** trichomes showing variations in terminal regions and apical cells. Scale bars: 10 µm for b-h; 2 cm for a.

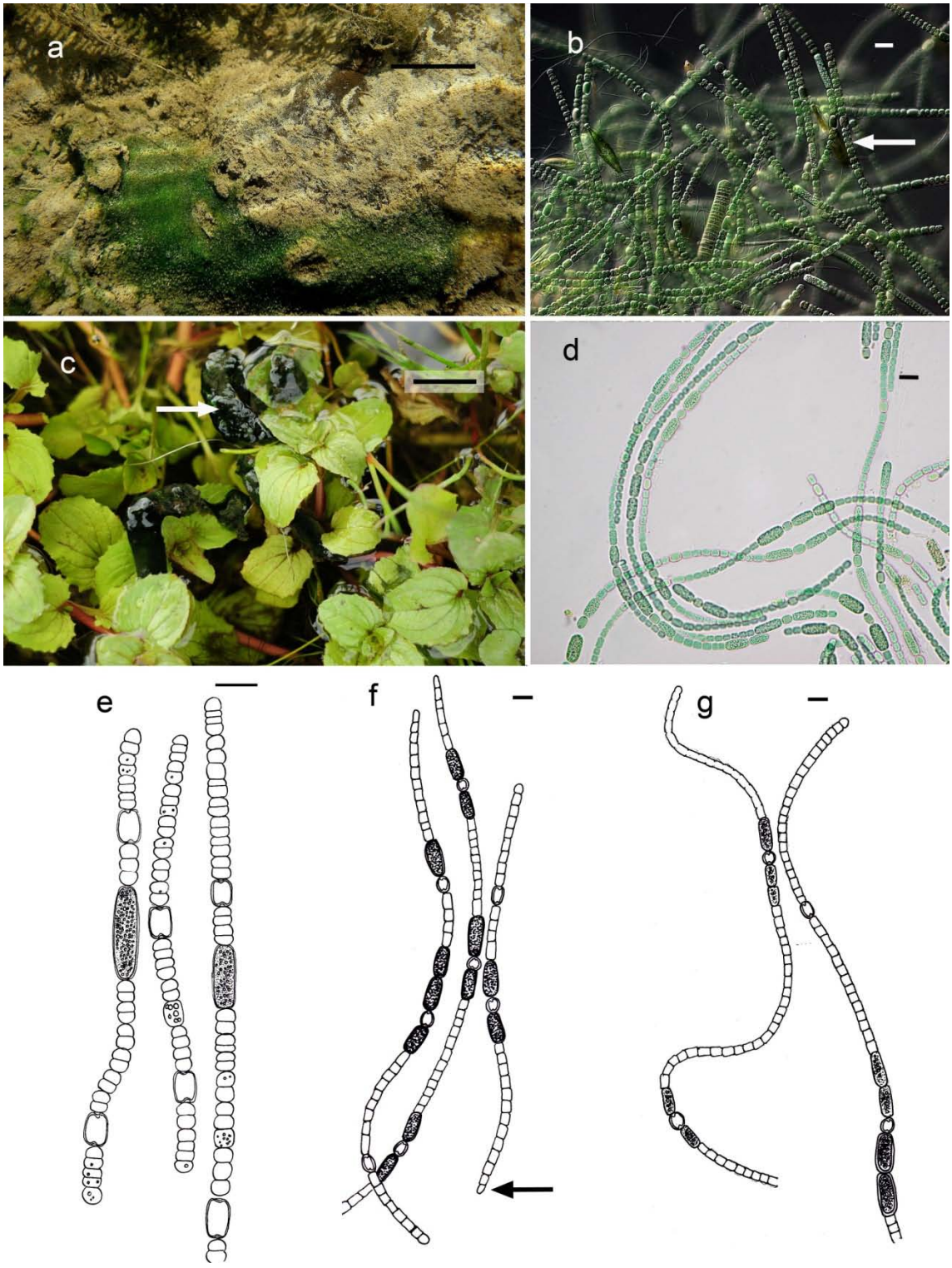

Figure 3.22: ***Anabaena*** cf. ***inaequalis***: field specimens, **a,** softly mucilaginous blue-green mats epipelic on silt; field specimens, **b,** trichomes observed in mats; **e,** trichomes with apoheterocytic akinete development. ***Anabaena oscillarioides***: field specimens, **c,** blue-green, soft gelatinous colonies floating amongst aquatic angiosperms (arrow)**; d,** trichomes with paraheterocytic akinete development; **f**, trichome morphology showing slight tapering at the apices (arrow); cultures, **g,** trichomes lacking tapering apices. Scale bars: 10 µm for b, d, e-g; 2 cm for a, c.

Anabaena oscillarioides Bory 1822
Whitton (2011): p124, pl. 25F (p123). Geitler (1932): p886, fig. 567. Desikachary (1959): p417, fig. 7, pl. 71 (p394)

Description: Colonies in field blue-green, softly gelatinous (Fig. 3.22c). Trichomes bright blue-green, long, straight to slightly flexuous, very slightly tapering towards apex, 4.0-6.0 µm wide, distinctly constricted at cross walls (Fig. 3.22d). Cells barrel-shaped, mostly longer than wide, 3.8-7.5 µm long. Apical cell rounded to slightly conically rounded. Heterocytes spherical or slightly elongated, 5.0-6.0 µm wide, 5.0-7.0 µm long. Akinetes cylindrical with smooth outer wall, brownish, develop adjacent to heterocytes, 7.0-10.0 µm wide, 15.0-25.0 µm long (Fig. 3.22f). Sheath absent.

Colonies on agarised culture medium bright blue-green, softly mucilaginous. Trichome morphology similar to field material except for absence of tapering at apices (Fig. 3.22g).

Occurrence: Entangled amongst aquatic angiosperms close to the banks in unshaded fourth order stream at site 57.

Remarks: Field specimens differ from *A. oscillarioides* (Whitton, 2011; Geitler, 1932; Desikachary, 1959) in the tapering trichomes and apical cell shape. Several varieties have been recognised based on features such as akinete size and apical cell shape (Whitton, 2011). Akinete length of Kaituna specimens falls in the lower end of the given range (20.0-40.0 µm long). Shorter akinetes have been reported (Parukutty, 1939)

Previous records suggest standing freshwater and temporary pools as the favoured habitat of *A. oscillarioides* (Whitton, 2011; Desikachary, 1959).

Cylindrospermum cf. ***muscicola*** (Kützing 1843) Bornet et Flauhault 1886
Whitton (2011): p131, pl. 27D (p132). Geitler (1932): p822. Desikachary (1959): p366, pl. 65, fig. 3 (p365)

Description: Mats in field bright blue-green, softly mucilaginous (Fig. 3.23a). Trichomes blue-green, long, flexuous, 3.0-4.0 µm wide distinctly constricted at cross walls (Fig. 3.23c, f). Cells barrel-shaped, shorter to longer than wide, 3.8-7.5 µm long. Heterocytes 3.8-5.0 µm wide, 6.3-12.5 µm long. Akinetes single, elongated oval, usually adjacent to terminal heterocytes (Figs. 3.23d,e), rarely distant from heterocytes (Fig. 3.23f), 10.0-12.0 µm wide, 18.0-20.0 µm long, with smooth golden brown wall, (Fig. 3.23g). Sheath absent.

Occurrence: Epipelic on sediment in slow flow unshaded fourth order stream at site 56.

Remarks: Specimens differ from *C. muscicola* (Whitton, 2011; Desikachary, 1959) in longer vegetative cells and heterocytes (4.0-5.0 µm and 5.0-7.0 µm long) respectively. Akinete development within the trichome distant from the heterocytes has not been reported for *C. muscicola*.

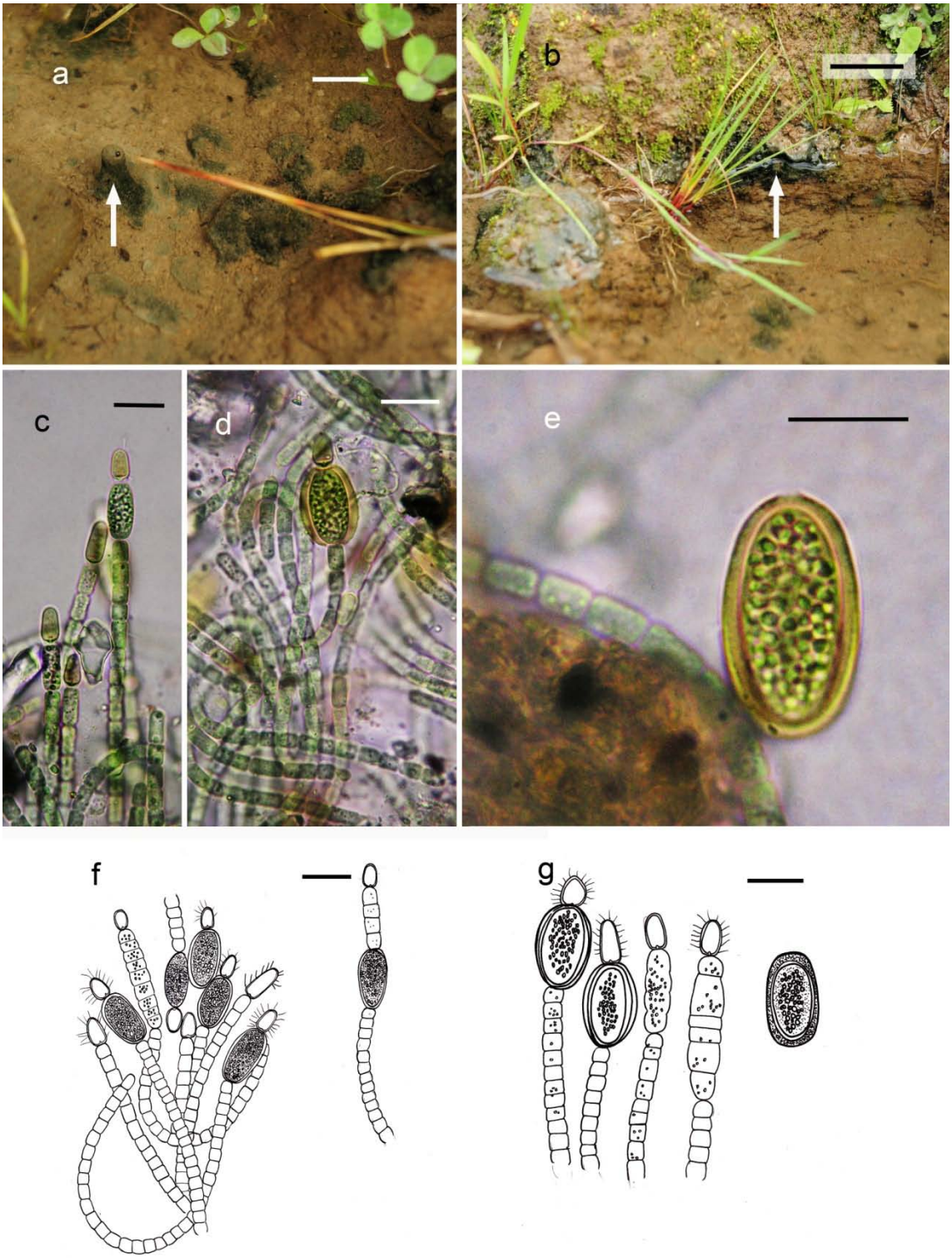

Figure 3.23: ***Cylindrospermum*** cf. ***muscicola*****:** field specimens, **a,** bright blue-green mats, epipelic on silt in slowly flowing shallow water (arrow); **b,** mats on moist soil slightly above water level (arrow); **c, d,** trichome morphology with akinete development adjacent to terminal heterocyte; **e,** morphology of the mature akinetes with golden-brown thick outer wall; **f,** variation in heterocyte shape, unusual development of an akinete distant from terminal heterocyte; **g,** akinete development by the fusion of several vegetative cells. Scale bars: 5 µm for c, d, f-g; 20 µm for e; 2 cm for a-b.

Previous collections from moist soils and standing waters (Whitton., 2011), streams (Sheath and Cole, 1996), rice fields (Desikachary and Indian Council of Agricultural, 1959; Pereira *et al*., 2005) and taro field (Evenhuis and Eldredge, 2012).

Nostoc verrucosum (Vaucher 1803) Bornet et Flahault 1886
Whitton (2011): p142, pl. 4B; Geitler (1932): p854, fig. 542; Desikachary (1959): p388, pl. 70, fig. 1 (p386)

Description: Colonies in field initially spherical or hemispherical, yellowish-brown, firmly gelatinous, 0.5-1.0 cm wide; subsequently developing into irregular shapes, 2.0-3.0 cm wide with folds and corrugations, becoming soft, torn and usually blackish-brown (Figs. 3.24a, e). Trichomes blue-green, flexuous, densely entangled towards periphery, slightly less so in centre (Fig. 3.24c, f). Cells barrel-shaped, shorter than wide, 3.0-3.5 µm wide. Heterocytes spherical, 6.0 µm diam.. Akinetes, ellipsoidal, colourless, 6.0 µm wide, 8.0 µm long, with smooth outer wall (only one observed), Mucilage diffluent, thick, yellow- brown towards periphery, hyaline in centre of colony.
Occurrence: Epilithic in unshaded fourth order stream.
Remarks: Colony morphology, trichome dimensions and presence or absence of akinetes are the key feature for *Nostoc* identification (Whitton, 2011). Specimens fit the description of *Nostoc verrucosum* (Geitler, 1932; Desikachary, 1959; Whitton, 2011).

Previously recorded from streams (Geitler, 1932a; Desikachary, 1959; (Sabater and Muñoz, 2000); Whitton, 2011) and moist soils (Desikachary, 1959).

Nostoc sp. 1
Whitton (2011): p137. Komárek and Anagnostidis (1989): p292, 306

Description: Colonies in field spherical and hemispherical, green, firmly mucilaginous, less than 0.5 cm wide; forming approximately circular, dense groups 5.0-7.0 cm in diam. (Figs. 3.24d, g). Trichomes blue-green, flexuous. Cells barrel-shaped, shorter than wide, 5.0-7.5 µm wide. Heterocytes spherical to ellipsoidal, 6.0 µm diam.. Akinetes not observed. Mucilage diffluent, hyaline.
Occurrence: Epilithic in unshaded fourth order stream.
Remarks: Colony shape of the present material did not conform to any species previously described (Geitler, 1932; Desikachary, 1959; Whitton, 2011).

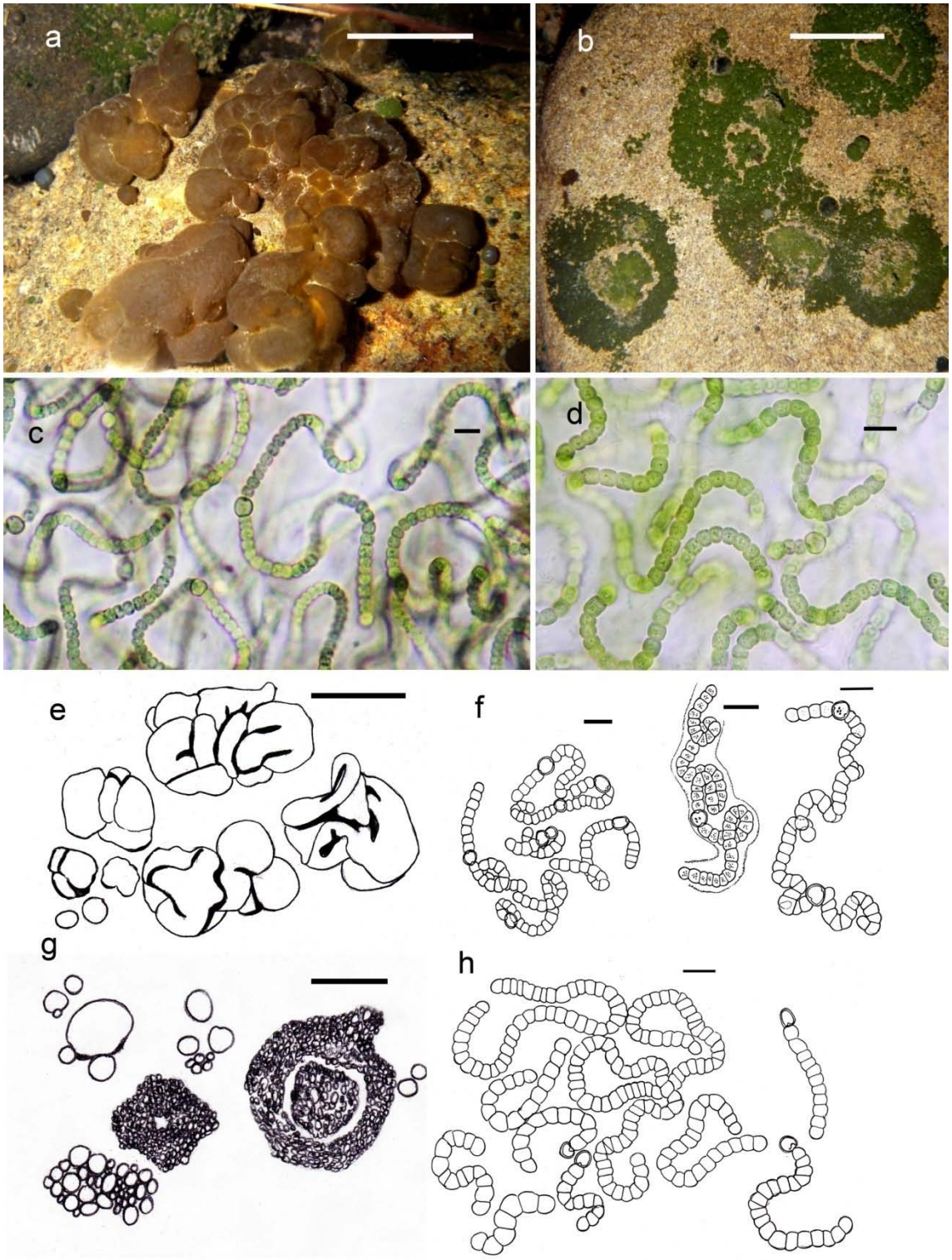

Figure 3.24: ***Nostoc verrucosum*****:** field specimens, **a,** yellowish-brown firmly mucilaginous colonies with folds and corrugations; **e**, variation in colony morphology; c, f**,** trichome flexuous with short cells. ***Nostoc*** **sp. 1:** field specimens, **b, g,** densely aggregated small firmly mucilaginous colonies forming circular outline; **d, h,** trichome wide with barrel shaped cells. Scale bars: 5 µm for c, d, f, h; 10 cm for a,b, e, g .

Nostoc sp.2
Whitton (2011): p138. Komárek and Anagnostidis (1989): p292, 306

Description: Colonies in field green, amorphous, softly mucilaginous (Fig. 3.25a, e). Trichomes blue-green, long, flexuous, densely entangled in the centre, slightly less so towards the periphery. Cells barrel-shaped, shorter than wide, 3.8-5.0 µm wide (Fig. 3.25f). Heterocytes spherical, 5.0-6.2 µm diam. Akinetes not observed. Mucilage firmhyaline.

Colonies on agarised culture media spherical, dark blue-green, to ca. 2.0 cm diam. (Fig. 3.25b). Trichomes more densely aggregated in colony center, morphology similar to field material apart from fewer heterocytes (Fig. 3.25c, d, g).

Occurrence: Entangled amongst aquatic angiosperms in unshaded fourth order stream at site 57.

Remarks: Seven morphospecies have softly mucilaginous or ill-defined colonies (Whitton, 2011). Five of these (*N. carneum*, *N. linckia*, *N. piscinale*, *N. spongiaforme* and *N. coeruleum*) have floating colonies usually occurring among leaves of macrophytes. The first four of these are all distinguished on the basis of akinete size and shape (Whitton, 2011). The lack of akinetes in Kaituna specimens prevented comparison with these morphospecies. Akinetes have not been described for *Nostoc coeruleum* and it has distinctly wider cells (5.0-7.0 µm) than Kaituna specimens.

Trichormus cf. ***variabilis*** (Bharadwaya) Komárek et Anagnostidis 1989
Whitton (2011): p125, pl. 24J (p121). Geitler (1932): p876, fig. 558 (877). Desikachary (1959): p410, pl. 71, fig. 5 (p394)

Description: Mats in field blue-green, mucilaginous. Trichomes bright blue-green, long, straight to slightly flexuous, tapering towards apical cell (1.6-2.0 µm wide), intercalary cells 3.8-5.0 µm wide, distinctly constricted at cross walls. Cells barrel-shaped, slightly longer or shorter than wide, 2.5-6.3 µm long. Apical cell conical. Heterocytes spherical, 5.0-6.3 µm wide diam.. Akinetes in short or long chains, first forming equidistant between two heterocytes and then further akinetes developing from adjacent vegetative cells (apoheterocytic), ellipsoidal, colourless or yellow-brown, with smooth outer wall, 6.2-7.5 µm wide, 7.8-11.3 µm long (Fig. 3.25i). Sheath absent.

Colonies on agarised culture medium bright blue-green, mucilaginous. Trichome morphology similar to field material apart from being wider (6.3 µm) and heterocytes being longer (Fig. 3.25h).

Occurrence: Epipelic on silt in unshaded third order stream.

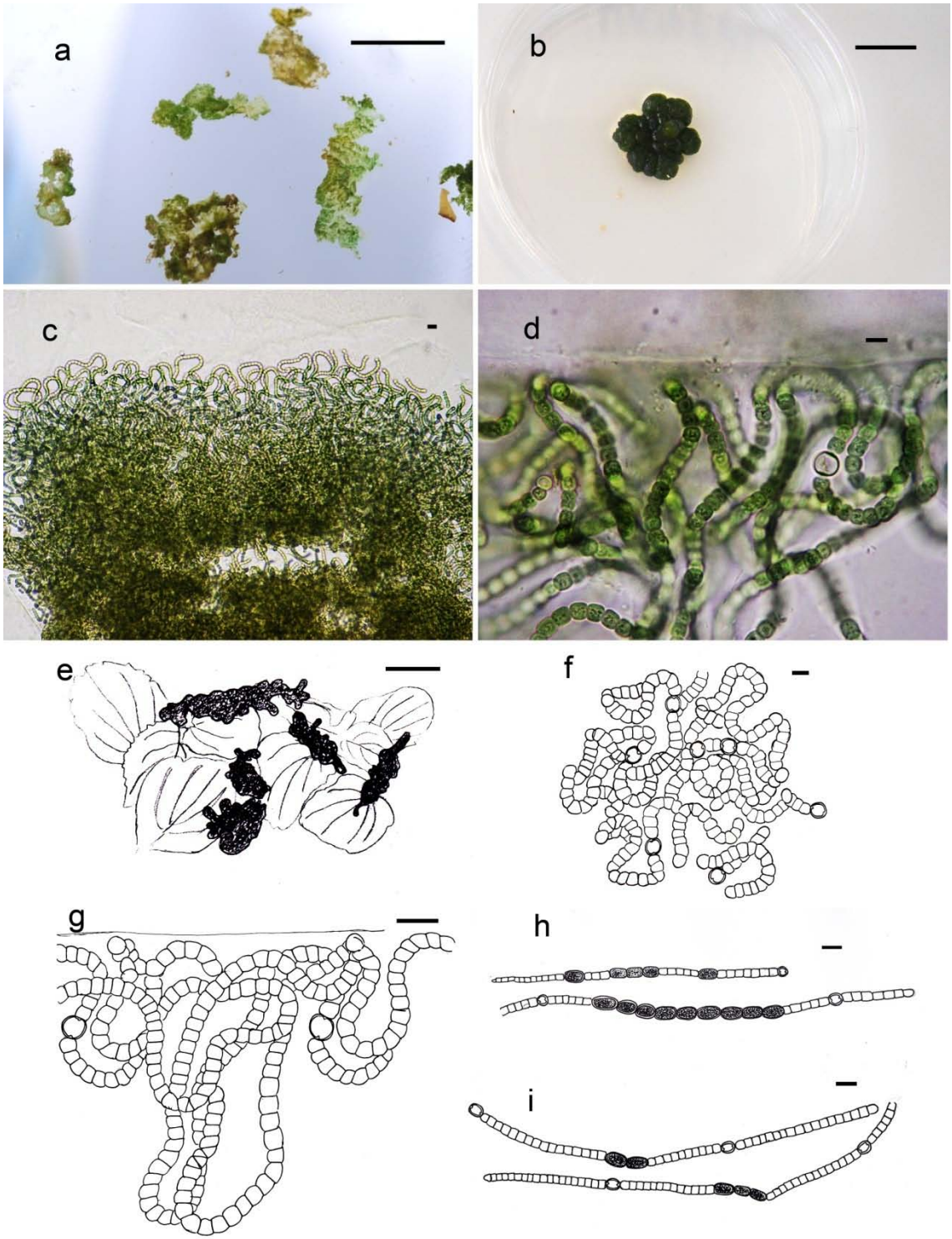

Figure 3.25: field specimens, ***Nostoc*** sp. 2: **a, e,** morphology of colony removed from amongst aquatic angiosperms**; f,** flexuous trichomes with short cells and hemispherical heterocytes; cultures, **b,** aggregate of small spherical dark blue-green colonies, firmly mucilaginous; **c, d, g,** typical arrangement of trichomes, denser towards the centre. ***Trichormus*** cf. ***variabilis***: field specimens, **h**, trichome tapered towards one end with apoheterocytic akinete development; cultures, **i,** as h. Scale bars: 5 µm for c, d, f-i; 2 cm for a, b, e.

Remarks: *Trichormus* is characterised by apoheterocytic development of akinetes (Komárek et Anagnostidis, 1989). Specimens differ from *T. variabilis* (Whitton, 2011; Getiler, 1932; Desikachary, 1959) in having tapered trichomes.

Previous collected from very moist soils (Desikachary, 1959; Komárek, 1992; (Hrčková *et al.*, 2010); Whitton, 2011) and stagnant freshwater (Gladkikh *et al.*, 2008).

Family **Rivulariaceae**

Calothrix braunii Bornet et Flahault 1886
Whitton, (2011): p128. Geitler (1932): p606, fig. 381. Desikachary (1959):p535, pl. 114, fig.3 (p541).

Description: Field specimens are frequent components of black crust. Trichomes blue-green, long, straight to slightly flexuous, often basally swollen and bent, 7.0-10.0 µm wide tapered to hyaline hairs, 1.0 µm wide, at apex, distinctly constricted at cross walls (Fig. 3.26a). Cells barrel-shaped, mostly shorter than wide, 1.6-4.8 µm long (Fig. 3.26d). Heterocytes hemispherical, basal, 4.0-8.0 µm wide, 4.0-5.0 µm long. Sheath thin, firm, hyaline, without lamellations.
Occurrence: Epilithic in unshaded third and fourth order streams. Only those in the latter had terminal hyaline hairs.
Remarks: Specimens differ from *C. braunii* (Desikachary, 1959) in the widened sheath. Trichome width falls within the low end of the given range. Hyaline hairs develop in response to phosphorus limitation (Whitton, 2011).

Previous records from streams (Uher and Kovácik, 2002; Whitton, 2011), on a calcareous wall (Uher, 2007) and in paddy fields (Desikachary, 1959).

Calothrix cf. ***epiphytica*** West et G.G. West 1897
Geitler (1932): p606. Desikachary (1959):p543. Whitton (2011): p129, pl. 26F (p127).

Description: Field specimens attached to colonies of *Nostoc.* Filaments solitary or in small groups. Trichomes blue-green, long, straight to slightly flexuous, 4.0-7.5 µm wide at the base, 3.0-5.0 µm wide in centre, tapered to a hyaline hair less than 1.0 µm wide, , constricted at cross walls (Fig. 3.26e). Cells barrel-shaped, mostly shorter than wide, 1.3-5.0 µm long, but longer than wide in apical parts. Heterocytes hemispherical, basal, 5.0-7.5 µm wide, 3.0-6.5 µm long. Sheath thin, firm, hyaline.
Occurrence: Epiphytic in unshaded fourth order stream.

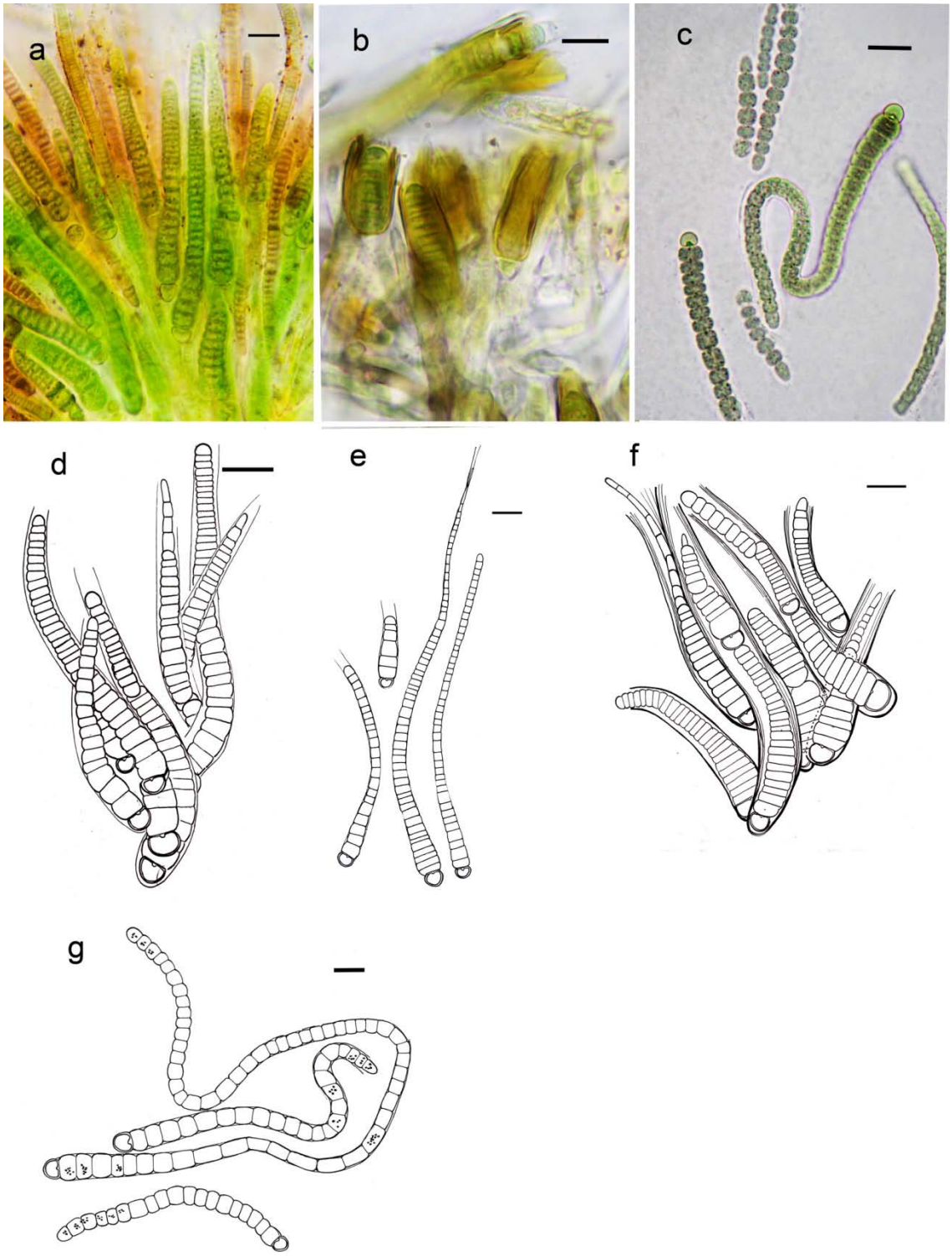

Figure 3.26: ***Calothrix braunii*****:** field specimens, **a, d,** trichomes with more or less isodiametric cells at the base and distinctly shorter cells towards the apex, apical cells more or less elongated. ***Calothrix parietina*****:** field specimens, **b,** short filaments with distinct yellowish-brown sheath; **f,** filaments with short cells and distinctly lamellated sheath. ***Calothrix*** sp.**:** cultures, **c, g,** trichomes with varying cell lengths, longer cells in central region of trichome. ***Calothrix*** cf. ***epiphytica*****:** field specimens, **e,** trichome narrow with cells shorter than wide, apical cells forming a hyaline hair. Scale bars: 10 µm for a-g.

Remarks: Specimens differ from *C. epiphytica* (Whitton, 2011; Desikachary, 1959) in wider trichome width and shorter cells.

Previous collections from freshwater as epiphytes on filamentous algae (Whitton, 2011), in rice paddy soils and attached to shells (Desikachary, 1959).

Calothrix parietina (Thuret 1875) Bornet et Flahault 1886
Geitler (1932): p604-605, fig. 380; Desikachary (1959):p538, pl. 107, fig.12 (p,) Whitton (2011): p129, pls. 26A-C (p127), 32H (p151).

Description: Field specimens are major components of black crust consisting of densely aggregated filaments. Trichomes blue-green, flexuous, 8.0-10.4 µm wide, tapereds towards the apex into hyaline hairs, 0.8-1.0 µm wide, often with false branches (Fig. 3.26f), distinctly constricted at cross walls. Cells barrel-shaped, distinctly shorter than wide towards base, isodiametric to longer than wide towards apex, 5.6-8.0 µm long. Heterocytes hemispherical, basal, 4.8-7.2 µm wide, 3.2-4.8 µm long. Sheath thick, lamellated, yellowish brown, often spread out towards the filament apex (Fig. 3.26b).
Occurrence: Epilithic in unshaded fourth order stream.
Remarks: Closely conforms with the species description provided by Whitton (2011).

Previously collected from stone surfaces in similar streams, also as epiphytes on aquatic and in garden ponds which are not too nutrient rich (Whitton, 2011).

Calothrix sp.
Whitton (2011): p126. Komárek and Anagnostidis (1989): p290-292

Description: Colonies on agarised culture medium blue green. Trichomes olive-green, flexuous, 6.3-8.8 µm wide at base, 5.0-6.6 µm wide in centre, tapered towards conical apical cell, distinctly constricted at cross walls (Fig. 3.26g). Cells generally barrel-shaped, distinctly shorter than wide towards the base, 3.8-5.0 µm long, sometimes longer than wide in center, 7.5-10.0 µm long (Fig. 3.26c). Heterocytes hemispherical, basal, 5.0-7.5 µm wide, 5.0-6.3 µm long. Sheath thin, firm, hyaline.
Remarks: Specimens differ in morphology from other *Calothrix* morphospecies described in the field material. Specimens did not conform to any species descriptions in Whitton (2011).

Dichothrix sp.
Whitton (2011): p131. Komárek and Anagnostidis (1989): p290-292

Description: Field specimens form black, small (ca. 1.0 mm) crust consisting of densely aggregated filaments. Trichomes dark blue-green, flexuous, tapered towards conical apical cell, often with false

branches (Fig. 3.27a), 6.0-8.0 µm wide at base, 3.0-4.0 µm wide at apex, distinctly constricted at cross walls. Cells barrel-shaped, 2.0-5.5 µm long, generally distinctly shorter than wide but longer at base. Heterocytes subspherical, basal, 4.0-6.0 µm wide, 2.0-4.8 µm long. Sheaths enclose individual trichomes and clusters of trichomes, thick, yellowish towards the apex (Fig. 3.27g).

Occurrence: Epilithic in unshaded second and third order streams.

Remarks: Specimens did not conform to any species descriptions in Whitton (2011).

Rivularia sp. 1

Whitton (2011): p142. Komárek and Anagnostidis (1989): p289

Description: Colonies in field are dominant component of black, distinctly circular in outline, 1 - 2 mm diam., flat to slightly raised crusts (Fig. 3.27b). Filaments densely packed. Trichomes blue-green, short, constricted at cross-walls, 4.0-6.0 µm wide towards base (Fig. 3.27c), tapered towards rounded to slightly elongated apical cell, 2.0–3.0 µm wide (Fig. 3.27e). Cells isodiametric to shorter than wide, 1.0-5.0 µm long. Heterocytes basal, subspherical 4.0-5.0 µm wide. Sheath thin, firm, hyaline.

Occurrence: Epilithic in unshaded fourth order stream. Colonies of *Rivularia* are mixtures of species 1 and 2.

Remarks: See remarks for species 2.

Rivularia sp. 2

Whitton (2011): p142. Komárek and Anagnostidis (1989): p289

Description: Colonies are frequent components of crusts as for *Rivularia* sp. 1 (Fig. 3.27b). Filaments densely packed. Trichomes blue-green, long, constricted at cross-walls, 6.0 - 8.0 µm wide towards base (Fig. 3.27d), tapered towards long, apical, multicellular, hyaline hairs, 1.0 - 2.5 µm wide (Fig. 3.27f). Cells isodiametric to shorter than wide, 2.5-7.5 µm long. Heterocytes basal, subspherical, 5.0 - 6.0 µm wide. Sheath thin, firm, hyaline.

Occurrence: Epilithic in unshaded fourth order stream. Colonies of *Rivularia* are mixtures of species 1 and 2.

Remarks: *Rivularia* spp. 1 and 2 co-occur in the same colony. Both differ from morphospecies with similar mature colonies described by Whitton (2011) in their lack of calcification. Some individual colonies of *Rivularia* have been reported to be phenotypically and genotypically heterogenous making it difficult to assign them to a particular species (Berrendero *et al.*, 2008).

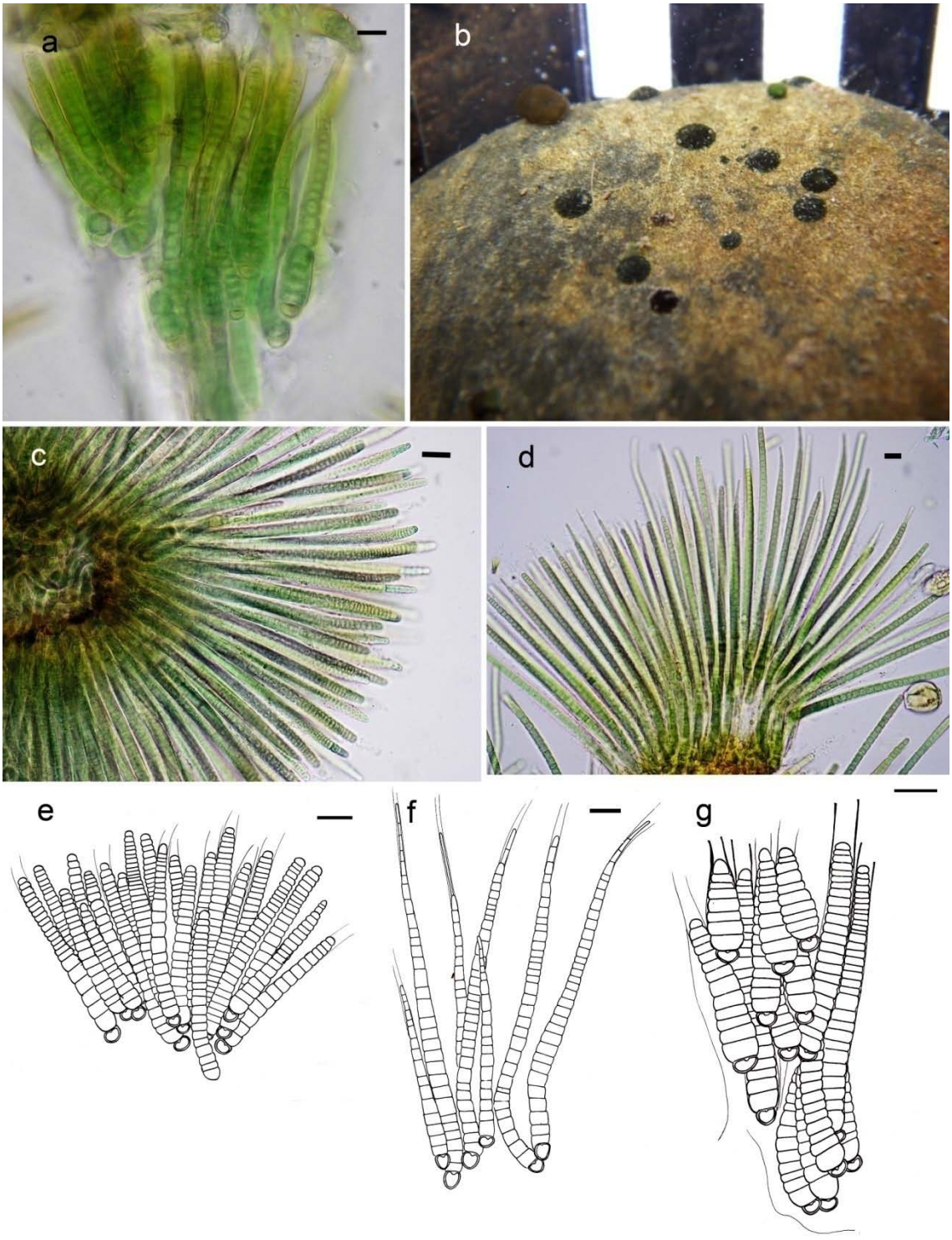

Figure 3.27: ***Dichothrix*** sp.**:** field specimens, **a**, dense aggregates of filaments with pigmented sheaths towards the apices; **g,** trichomes with broad cells at the base, tapering towards the apex, clustered within a common sheath. ***Rivularia*** spp.**:** field material **b,** black, circular, slightly raised colonies consisting of species 1 and 2. ***Rivularia*** sp. **1**: field material; **c,** filaments densely aggregated in colonies; **e,** short filaments with distinctly shorter cells towards the apex, apices not elongated into fine hairs. ***Rivularia*** sp. **2**: field specimens; **d**, filament densely aggregated in colonies; **f,** trichomes with cells towards the apices longer than wide and forming hyaline hairs. Scale bars: 10 µm for for a, c-g; 5 mm for b.

3.4 Discussion

3.4.1 Cyanobacteria in New Zealand flowing waters

The 56 morphospecies identified in this study have increased the known number of benthic cyanobacteria in New Zealand lotic habitats. Biggs (1990) found cyanobacteria to be a minor component of periphyton and numerous other studies have reported very low species richness of periphytic cyanobacteria (Collier and Winterbourn, 1990; Jaarsma *et al.*, 1998; Quinn *et al.*, 1997a; Suren *et al.*, 2003). Some (Biggs and Gerbeaux, 1993; Jowett and Biggs, 1997) recorded none. In studies lacking a specific focus on cyanobacteria, records are made of only prominent cyanobacteria that dominate periphyton (Biggs, 1990; Biggs and Smith, 2002; Francoeur *et al.*, 1998).

Phormidium is the genus most commonly recorded in New Zealand flowing waters (Biggs and Price, 1987; Biggs, 1990; 2000b). It is also widespread in flowing waters worldwide (Branco *et al.*, 2001; Filkin *et al.*, 2003; Sheath and Cole, 1992; Sheath and Cole, 1996). In New Zealand, periphytic *Phormidium* mats have been the focus of recent studies due to an ability to form toxins (Hamill, 2001; Heath *et al.*, 2010; Wood *et al.*, 2007;2012). Thirty *Phormidium* morphospecies from freshwater are known for New Zealand (Broady and Merican, 2012). *Phormidium autumnale* has attracted detailed study (Wood *et al.*, 2007; Heath *et al.*, 2010) whilst other morphospecies have been neglected. Previous records of mat-forming genera include *Lyngbya* (Biggs, 1990; Collier and Winterbourn, 1990; Quinn *et al.*, 1997; Francoeur *et al.*, 1998; Jaarsma *et al.*, 1998), *Oscillatoria* (Jaarsma *et al.*, 1998; Heath *et al.*, 2010), *Nostoc* (Heath *et al.*, 2010) and *Planktothrix* (Wood *et al.*, 2010).

The mat community of the study site comprised 16 morphospecies from the Oscillatoriales and Nostocales. Seven are new records for New Zealand (Table 3.1). Macroscopically, each of 14 of these 16 morphospecies formed a unique mat type. Additionally, two and three mat types respectively were formed by *P. autumnale* and *P. uncinatum*. These differed in pigmentation and their location within Kaituna River. The differences in pigmentation could be due to differences in light intensity but there is no data to support this hypothesis. In his review of the physiological ecology of cyanobacteria, Stal (1995) noted that many mat types are dominated by a single species but the many factors that might be responsible for this are little understood. Ability to utilize nutrients more efficiently, adaptability to changes in light intensity and water flow rates, faster growth, and perhaps morphological stability could possibly lead to the dominance of the more resistant morphospecies.

Morphospecies of *Phormidium* dominated most mat types in this study. Identification of morphospecies within this genus is problematic (Komárek and Anagnostidis, 2005; Marquardt and Palinska, 2007; Palinska *et al.*, 2011). Six out of seven morphospecies (*P. autumnale*, *P.* cf. *bekesiense*, *P. inundatum*, *P.* cf. *irriguum*, *P.* cf. *subfuscum* and *P. uncinatum*) have been successfully grown in unialgal cultures. Only *P. chalybeum* was not isolated.

For four of the cultured morphospecies, field specimens and cultured strains were very similar in morphology. This has been reported to be the case in other studies (Palinska and Marquardt, 2008; Hašler *et al.*, 2012). Marked differences in trichome width were observed only in *P*.cf. *bekesiense* and *P*. cf. *subfuscum.* This could be due to differences in light and nutrient availability between the field and cultures as suggested by Marquardt and Palinska (2007). Morphospecies identifications of all the six *Phormidium* morphospecies have been confirmed by molecular phylogenetics (Chapter 4).

Collier and Winterbourn (1990) reported a *Chamaesiphon*-like alga whilst using scanning electron microscopy to view epilithic crusts from circumneutral streams. This genus is widespread in New Zealand flowing waters (Biggs and Kilroy, 2000). Other previously recorded components of crusts include *Amphithrix* (possibly a misidentification of *Homoeothrix;* Francoeur *et al.* 1998), *Calothrix, Tolypothrix* (Francoeur *et al.* 1998; Biggs and Smith, 2002) and *Dichothrix* (Biggs and Kilroy, 2000). Only *Tolypothrix* was not observed in Kaituna crusts but the reason for this absence is unclear.

Twenty-two morphospecies of crust forming morphotypes were recorded of which 16 are new records for New Zealand (Table 3.1). A representative of Entophysalidaceae, *Chlorogloea* cf. *microcystoides,* has been recorded for the first time in New Zealand. Crust-forming morphospecies in this study almost entirely comprise rarely recorded and poorly known genera. This reflects a general lack of studies on crust communities worldwide despite a recent rise in interest (Johansen *et al.*, 2011; Rott, 2008; Sant'Anna *et al.*, 2011). Crusts are often inconspicuous, being thin and often of similar colour to their substrata. This has resulted in them being easily overlooked.

Attempts were made to establish unialgal cultures for all crust components. Only *Heteroleibleinia fontana* was grown successfully. The morphology of field specimens differed from that of cultured strains, particularly in colony appearance. This supports the suggestion of Whitton (2011) that cultures always need to be compared with field specimens to avoid erroneous identifications. Strains of *H. fontana* were included in molecular phylogenetic analyses (Chapter 4).

Nostoc has been reported as the most widespread gelatinous colony forming periphyton in New Zealand (Biggs and Kilroy, 2000) but its occurrence has not been well-documented. Five morphospecies formed gelatinous colonies in this study. Two are new records for New Zealand (Table 3.1). Two morphospecies of *Nostoc* could not be confidently identified using available literature and require further study. Molecular phylogenetics did not aid in their species identification (Chapter 4**).**

Periphyton assemblages in New Zealand are reported to be different from those in other parts of the world (Biggs, 1990). In a survey of streams in mountain and hill country of the South Island, Broady and Ingerfeld (1991) reported a new species, *Placoma regulare.* This has been recorded only in New Zealand. Extensive surveys of rivers and streams around New Zealand are recommended to reveal a possibly rare and endemic flora.

Epiphytic cyanobacteria occurred in high abundance on filamentous chlorophytes and rhodophytes at the study site. Seven morphospecies were recorded of which four are new records for New Zealand (Table 3.1). The only other record of epiphytic cyanobacteria in New Zealand rivers and streams is of the occurrence of young colonies of *Placoma regulare* on liverworts (Broady and Ingerfeld, 1991) .

3.4.2 Comparison with cyanobacterial floras in flowing waters outside New Zealand

Many cyanobacteria morphospecies have been reported as cosmopolitan (Komárek and Anagnostidis (1999; 2005). The apparently universal distribution, typical of microorganisms, has been suggested to result from massive population sizes and efficient passive dispersal over large areas assisted by small cell size (Tom and Bland, 2004). Cosmopolitan distribution in a number of cyanobacteria morphospecies have been confirmed using molecular markers (Jungblut *et al.*, 2010; van Gremberghe *et al.*, 2011). Conversely, clear phylogeographic structuring (Zwirglmaier *et al.*, 2008) and endemism (Taton *et al.*, 2003) are also evident in cyanobacteria.

No flora recorded from any other region worldwide is identical to the present flora but some do contain one or more morphospecies found in the present study. Comparison with these floras is useful for an insight into the geographical distribution of the recorded morphospecies but this is a tentative comparison as taxonomic uncertainties and under-sampling is inevitable as outlined below. A notable feature is the small proportion of morphospecies common to the present study site and to each of the following studies given in Table 3.2.

Phormidium autumnale and *N. verrucosum* are the most commonly shared morphospecies between the present and other studies (Table 3.2). *Phormidium autumnale* has been reported as the most widespread morphospecies worldwide (Geitler, 1932) but morphological variability has resulted in problematic identifications (Palinska and Marquardt, 2008; Strunecký *et al.*, 2010). Descriptions of the morphospecies from numerous collections do not conform to the concept of *P. autumnale* as described by Komárek and Anagnostidis (2005). This has resulted in unreliable identifications and has deterred investigations of its biogeographic distribution. *Nostoc verrucosum* has also been reported as cosmopolitan (Skinner and Entwisle, 2001; Whitton, 2011). Unlike *P. autumnale*, mature colony morphology, texture and habitat preferences of *N. verrucosum* are diacritical characteristics separating it from morphologically similar *N. commune* (Skinner and Entwisle, 2001; Whitton, 2011). The validity of these characteristics in identification has been supported by molecular phylogeny of *N. verrucosum* from this study (Chapter 4).

Table 3.2: Morphospecies recorded from this study compared with records of similar morphospecies from flowing waters elsewhere. *This study had few sampling locations but sampled over a period of 12 years.

	Region	No. of morphospecies found	No. in common	No. of locations examined	Morphospecies in common
Present study	New Zealand	56	-	100	
Sherwood (2006)	Hawaiian Islands	57	6	167	*Phormidium autumnale, P. inundatum, Leptolyngbya foveolarum, Oscillatoria limosa, Calothrix braunii* and *N. verrucosum*
Filkin *et al.* (2003)	Hawaiian Islands	19	1	23	*Oscillatoria limosa*
Sheath and Cole (1992)	North America	62	5	1000	*Phormidium inundatum, P. uncinatum, Homoeothrix juliana, O. limosa* and *Nostoc verrucosum*
Sheath *et al.* (1996)	North America	32	2	150	P. *autumnale,* and *N. verrucosum*
Schneider and Lindstrøm (2009)	Norway	56	6	328	*Chamaesiphon amethystinus, C. incrustans, C. subglobosus, P. autumnale, Calothrix braunii* and *N. verrucosum*
Lindstrøm *et al* .(2004)	Norway	40	5	7 *	*Chamaesiphon confervicolus, C. subglobosus, P. autumnale, C. braunii* and *N. verrucosum*
Barinova and Nevo.(2010)	Lebanon	45	4	21	*Chamaesiphon incrustans, Homoeothrix juliana, P. autumnale* and *P. uncinatum*

Fifty-six morphospecies have been recorded in the present study from 100 locations within a single catchment. This diversity is similar to that of previous studies with diverse sampling localities as listed above (Table 3.2). However, there is a limitation to this comparison. These previous broad-scale surveys lacked focus on cyanobacteria hence their true diversity could have been under-estimated. A more cyanobacteria specific survey was conducted by Douterelo *et al.* (2004). They recorded 40 morphospecies of just epilithic cyanobacteria from a total of only six sites in three Spanish rivers in comparison with 36 epilithic morphospecies in the present study. As noted above for other studies, there is little similarity in morphospecies between the two studies. Just two of their records, *Calothrix braunii* and *C. parietina*, were recorded in the present study.

Comparison would be most valid with other studies investigating cyanobacterial diversity of similarly sized catchments. Such studies are scarce globally. In Spain, Perona and Mateo (2006) recorded 13 morphospecies of cyanobacteria from Alberche River while Loza *et al.* (2013a) recorded 17 morphospecies from Guadarrama River. There is no overlap in the morphospecies recorded by Perona and Mateo (2006) with those from the present study. Only *P.autumnale* was found in this study and by Loza *et al.* (2013). Both Perona and Mateo (2006) and Loza *et al.* (2013) investigated the diversity of epilithic cyanobacteria from less than 10 sampling sites within a single catchment. Thirty-six epilithic cyanobacteria have been recorded from 100 locations in the present study. It is difficult to assess whether the differences in numbers of morphospecies recovered is due to a real difference or just to different intensities of sampling.

Chlorogloea cf. *microcystoides* is the first representative of the Entophysalidaceae to have been recorded in New Zealand. It has a widespread distribution elsewhere. Originally recorded by Geitler (1932) from cold limestone Alpine streams, more recent accounts have included records from streams (Barinova and Nevo, 2010, Whitton, 2011; Schneider and Lindstrøm, 2009), in thermal springs (Economou-Amilli and Anagnostidis, 1981), an Antarctic lake (Starmach, 1995) and on stony walls (Hauer, 2007; Macedo *et al.*, 2009). Komárek and Anagnostidis (1999) expressed doubt about the identification of the morphospecies made from thermal springs and stony walls. The authors suggested that these records should be carefully reviewed.

Numerous studies have been conducted on epiphytic cyanobacteria in flowing waters worldwide (Dunn *et al.*, 2008; Gold-Morgan *et al.*, 1994; Lindstrøm *et al.*, 2004; Montejano *et al.*, 1997; Rodríguez *et al.*, 2011). No morphospecies found in those were recorded in the present study. Dunn *et al.* (2008) found 45 epiphytic morphospecies on the submersed aquatic macrophyte *Vallisneria americana* from lower St. John's River, Florida. The diversity recorded in the present study is far less with only seven morphospecies recorded. Although other macroalgae (*Vaucheria*, filamentous chlorophytes and rhodophytes) and vegetation (angiosperms, bryophytes) have been examined for

epiphytic cyanobacteria, this was not a major focus of the study and those occurring at low abundance could have been overlooked.

Epiphytic cyanobacteria on submerged macrophytes in eutrophic ponds in Saudi Arabia have recently been reported to include two toxic, microcystin-producing morphospecies, *Merismopedia tenuissima* and *Leptolyngbya boryana* (Mohamed and Al Shehri, 2010). Further floristic surveys of epiphytic cyanobacteria in New Zealand flowing waters would be valuable and their potential for toxin production requires investigation.

Twenty-nine morphospecies in this study (52% of the total) are new records for New Zealand. Almost half of these are rare and poorly known taxa worldwide. It is important to investigate the diversity of cyanobacteria at a similar intensity of sampling in other flowing waters in New Zealand in order to determine whether their floras differ in response to different environmental conditions.

Chapter 4

Molecular Phylogenetics of Selected Cyanobacteria

4.1 Introduction

4.1.1 The contribution of molecular phylogenetics to taxonomy of cyanobacteria

Molecular phylogenetics has had a great impact on cyanobacterial taxonomy. The use of two different nomenclatural codes, the Botanical and Bacteriological codes has caused considerable confusion and molecular phylogenetics is contributing to resolving of this. Two other significant problems are that identifications of many strains deposited in international culture collections rely solely on morphological traits while many nucleotide sequences found in public databases are from environmental DNA extracted from cyanobacteria that have not been observed microscopically (Rajaniemi *et al.*, 2005; Wilmotte and Herdman, 2001).

It is estimated that as many as 50% of cyanobacterial strains in culture collections have been identified incorrectly (Komárek and Anagnostidis, 1989). This is especially true for the Oscillatoriales, and at the lower taxonomic levels (genus and species), where there are significant problems in classification (Marquardt and Palinska, 2007; Palinska and Marquardt, 2008; Palinska *et al*., 2011; Strunecký *et al.*, 2010). The solution to the ever changing classification system and lack of a consensus phylogeny has been the focus of taxonomists for nearly 30 years (Komárek, 2006; 2010; Oren, 2004).

To resolve these issues, a polyphasic approach for species delimitation has been recommended in which conventional taxonomy is integrated with genetic characterization (Boutte *et al*., 2005; Lokmer, 2007; McGregor and Rasmussen, 2008; Zapomělová *et al.*, 2010; Sciuto *et al.*, 2012;).

Almost all traditional genera based on distinct morphological features have been confirmed by molecular phylogeny (Komárek, 2010). In the first molecular analysis, Giovannoni *et al.* (1988) showed Chroococcales and Oscillatoriales to be polyphyletic. All subsequent molecular analyses supported this observation (Litvaitis, 2002; Rajaniemi *et al.*, 2005; Schirrmeister *et al*., 2013). Pleurocapsalean species that form baeocytes were first thought to be monophyletic (Giovannoni *et al.*, 1988) but were subsequently found to be polyphyletic (Ishida *et al.*, 2001; Fewer *et al.*, 2002). Monophyly of heterocytic cyanobacteria reported by Giovannoni *et al,* (1988) is supported by subsequent studies (Nelissen *et al.*, 1996; Honda *et al.*, 1999; Litvaitis, 2002). However, in some molecular analyses (Fewer *et al.*, 2002; Tomitani *et al.*, 2006), only Stigonematales are usually resolved as monophyletic while Nostocales are often paraphyletic. It is apparent that molecular phylogenies produce conflicting assessments of relationships within the cyanobacteria.

4.1.2 Methods used in molecular phylogenetics

Comparison of nucleotide sequences of the 16S rDNA (the gene coding for the small-subunit ribosomal RNA) is currently the most common approach to phylogenetic classification of cyanobacteria (Giovannoni *et al.*, 1988; Neilan *et al.*, 1997; Honda *et al.*, 1999; Steindler *et al.*, 2005; Komarkova *et al.*, 2009; Heath *et al.*, 2010; Zapomělová *et al.*, 2011). In compliance with the Bacteriological Code, the 16S rDNA gene is used for identification and cataloguing of cyanobacteria (Castenholz, 2001). 16S rDNA is universal in bacteria and comparison of sequences allows recognition of genera for all major phyla (Clarridge, 2004). It is the basis for defining taxonomic groups in the second edition of Bergey's Manual of Systematic Bacteriology (Wilmotte and Herdman, 2001).

16S rDNA is one of the three genes that form the ribosomal RNA (rRNA) operons in bacteria (Iteman *et al.*, 2000). It contains about 1, 550 nucleotides that provide a large number of characters which allow statistically valid comparisons (Clarridge, 2004; Wilmotte, 1994). 16S rDNA is highly conserved in all bacteria suggesting evolution from a common ancestor (Wilmotte, 1994). This is assumed to be related to its critical function in protein synthesis (Clarridge, 2004).

There is a large and growing database of 16S rDNA sequences which is improving the strength of phylogenetic reconstructions (Nubel *et al.*, 1997). Over 90,000 sequences of 16S rDNA in GenBank are increasingly allowing a sequence from an unknown organism to be usefully positioned within a phylogeny (Clarridge, 2004). However, these sequences are of variable length and different regions of the gene have been analysed. This necessitates the study sequences to be aligned with other sequences to obtain useful data (Tindall *et al.*, 2010). If there is a short or no overlap between two sequences some analyses are not possible, e.g. those that are distance based, and others, e.g. parsimony based ones, will be inaccurate. Caution is also required when making phylogenetic decisions as incorrect names have often been applied to the species for which sequences have been lodged in GenBank (Komárek, 2010). This partly arises because GenBank does not allow the attachment of an unpublished name to a sequence.

The high level of conservation of the 16S rDNA gene sequence limits its use for determining closely related species, even when species are morphologically distinct (Fox *et al*., 1992; Moore *at al.*, 1997; Rosselló-Mora and Amann, 2001). As a result, many studies have turned to the 16S-23S rRNA internal transcribed spacer (ITS) region as a complementary analysis for identification at species level (Boyer *et al.*, 2001; Brown *et al.*, 2005; Iteman *et al.*, 2000; Novis and Visnovsky, 2011).

The ITS region is highly variable in both sequence and length, occasionally even between multiple copies within a single genome (Gugger *et al.*, 2002). It contains information for antitermination and rRNA folding and codes for up to two transfer RNA (tRNA) genes (Swingley *et al.*, 2008). In

cyanobacteria, three types of 16S-23S ITS sequence compositions have been identified (Boyer *et al.*, 2001). The most common composition by far, containing both $tRNA^{Ile}$ and $tRNA^{Ala}$, has been reported in members of *Synechococcus* (Tomioka and Sugiura, 1984), *Arthrospira* (Nelissen *et al.*, 1994), *Phormidium* (Novis and Visnovsky, 2011), *Anabaena* (Gugger *et al.*, 2002), *Nostoc* (Iteman *et al.*, 2000), *Trichodesmium* (Wilmotte *et al.*, 1994), *Calothrix* (Boyer *et al.*, 2001) and *Coloedesmium, Scytonema* and *Tolypothrix* (Boyer *et al.*, 2001; Novis and Visnovsky, 2011). Several examples containing only $tRNA^{Ile}$ include *Microcystis* (Novis and Visnovsky, 2011; Otsuka *et al.*, 1999), *Synechococcus* (Novis and Visnovsky, 2011) and *Spirulina* (Nelissen *et al.*, 1994; Novis and Visnovsky, 2011). The third composition shows a lack of tRNA genes and has been reported in members of *Nostoc* (Iteman *et al.*, 2000), *Nodularia* (Hayes and Barker, 1997), *Calothrix* and *Scytonema* (Boyer *et al.*, 2001).

16S-23S ITS has been successfully utilized for differentiating relationships at species and subspecies level in members of *Arthrospira* (Scheldeman *et al.*, 1999; Ballot *et al.*, 2004), *Microcystis* (Otsuka *et al.*, 1999), *Phormidium* (Marquardt and Palinska, 2007; Novis and Visnovsky, 2011; Sciuto *et al.*, 2012) and *Synechococcus*-like strains (Becker *et al.*, 2004).

Amplified fragment length polymorphisms (AFLP) (Vos *et al.*, 1995) have also been used for investigation of genetic relationships and diversity. It investigates polymorphisms associated with restriction sites in the genome as opposed to RFLP (restriction fragment length polymorphism) which is a length-based technique (Janssen *et al.*, 1996). The origin of the name AFLP is solely based on the similarity of the technique with RFLP analysis (Vos *et al.*, 1995). It utilises sites throughout the whole genome rather than specific genes. Genetic relationships amongst strains can be reflected by the variation in banding patterns. These banding patterns can be considered as genomic fingerprints that allow numerical analysis for the purpose of identification and characterization (Janssen *et al.*, 1996).

AFLP is repeatable and its sensitivity can be adjusted. The concept of AFLP was originally developed for plant-breeding purposes but it is now considered a universal method for fingerprinting DNA of any origin or complexity (Vos *et al.*, 1995). This method has been used effectively in investigating cyanobacterial taxonomy of highly related strains (Janssen *et al.*, 1996) and genetic diversity of cyanobacterial populations (Satish *et al.*, 2001; Oberholster *et al.*, 2005; Novis and Smissen, 2006).

The rapidly increasing number of complete genome sequences offers new possibilities for understanding cyanobacterial taxonomy, phylogeny and diversity (Nakamura *et al.*, 2003; Mulkidjanian *et al.*, 2006; Shi and Falkowski, 2008; Beck *et al.*, 2012). *Synechocystis* sp. PCC 6803 was thefirst cyanobacterium to be sequenced (Kaneko *et al.*, 1996). Since then, 35 complete sequences have become available (Nakao *et al.*, 2010) with estimated genome sizes varying from 1.6

x 10^6 bp in unicellular forms and up to 13.2 x 10^6 bp in filamentous forms (Castenholz, 1992; Iteman *et al.*, 2000; Shih *et al*., 2012).

One of the major findings of whole genome studies is the extent of horizontal gene transfer (HGT). HGT, potentially followed by gene duplication or loss, and recombination are significant events in evolution of prokaryotes (Raymond *et al.*, 2002; Gogarten and Townsend, 2005;). Cyanobacteria can show a high degree of HGT (Lodders *et al.*, 2005) and this makes it more difficult to construct meaningful phylogenies. However, there is a core of genes that remain closely associated and resistant to HGT (Shi and Falkowski, 2008). These core genes possibly permit separation of true phylogenetic signals from 'noise' (Shi and Falkowski, 2008). Unfortunately, the use of this method for phylogenetic purposes alone is still very expensive and impractical (Kauff and Büdel, 2011).

The use of molecular phylogenetics is recommended as the basic standard for cyanobacterial classification (Komárek *et al.*, 2009). 16S rDNA has proved very useful in supporting numerous traditional genera whilst also recognizing the heterogeneity of others (Komárek *et al.*, 2009). Correlation between morphology and 16S rDNA phylogenies has been shown in heterocytous cyanobacteria (Tomitani *et al.*, 2006) and in some genera of Oscillatoriales, including *Planktothrix* (Komárek, 2006), *Blennothrix* (Abed *et al.*, 2006), *Oscillatoria* (Luke Simmons *et al.*, 2008), *Trichodesmium* (Wilmotte and Herdman, 2001) and *Arthrospira* (Komárek, 2006). Prochlorophytes comprised of the genera *Prochlorococcus, Prochlorothrix* and *Prochloron* which lack phycobiliproteins and contain divinyl-chlorophylls *a* and *b* (Chisholm *et al.*, 1992; Hess *et al.*, 1996) were originally considered a unique group of oxyphototrophic prokaryotes. However, the monophyletic group of *Prochlorococcus* strains has been shown to be nested within paraphyletic assemblages of marine *Synechococcus* strains (Litvaitis, 2002; Swingley *et al.*, 2008) while *Prochloron* might be a close relative of the Pleurocapsales (Sanchez-Baracaldo *et al.*, 2005). Undoubtedly, cyanobacterial taxonomy requires continuing revision using polyphasic evaluation of the full range of characters recognisable both in nature and cultures (Komárek *et al.*, 2009).

4.1.3 Aims

Molecular genetic approaches have been used to investigate the following.

1. Phylogenetic relationships of five cultured strains (*Placoma regulare*, *Heteroleibleinia fontana*, *Nostoc verrucosum*, *Nostoc* sp. 1 and *Nostoc* sp. 2) using 16S rDNA.
2. Phylogenetic relationships of closely related strains of Oscillatoriales (*Phormidium autumnale*, *P.* cf. *bekesiense*, *P. inundatum*, *P.* cf. *irriguum*, *P. cf. subfuscum*, two strains of *P. uncinatum* and *Oscillatoria curviceps*) using 16S-23S ITS.
3. Comparison of the genetic diversity of *Nostoc verrucosum* populations collected within the study stream with that of this morphospecies collected from other streams in the same geographical region, using AFLP.

4.2 Methods

4.2.1 DNA extraction, PCR and sequencing

DNA was extracted from cultured strains (see section 3.3.2 in Chapter 3) of Chroococcales (*Placoma regulare*) and nine Oscillatoriales (*Heteroleibleinia fontana*, *Phormidium autumnale*, *P. cf. bekesiense*, *P. inundatum*, *P. cf. irriguum*, *P. cf. subfuscum*, two strains of *P. uncinatum*, *Oscillatoria curviceps*) and from field specimens of three morphospecies of *Nostoc* (*Nostoc verrucosum*, *N.* sp. 1, *N.* sp. 2). Extraction used Maxwell ®16 DNA purification kits according to the manufacturer's instructions. Samples were initially placed in the buffer provided by the manufacturer, heated to 65°C in a heat block and ground up manually to maximize extraction products. DNA was stored frozen at -20°C.

PCR amplifications of the partial 16S rDNA gene for all Chroococcales and Nostocales were performed using the combination of primers 2 (5'- GGG GGA TTT TCC GCA ATG GG - 3') and 3 (5'- CGC TCT ACC AAC TGA GCT A - 3') while the full 16S-23S ITS region was amplified for all Oscillatoriales using primers 1 (5'-CTC TGT GTG CCT AGG TAT CC-3')and 5 (5'-TGT AGC TCA GGT GGT TAG-3') of Boyer *et al.*(2001). For *Nostoc verrucosum*, 16S rDNA sequences were confirmed by repeat sequencing using the combination of primers designed by P. Novis (Landcare Research, Lincoln, NZ); Nostoc KF (5' – CTC CGT GCC AGC AGC CGC GG – 3') and Nostoc KR (5' – TCC TCC GGT TTG TCA CCG GC – 3'). Each 20 µL PCR reaction contained 11.5 µL of sterile water, 2.0 µL of 10 X buffer (Invitrogen), 2.5 µL of dNTPs (2.5 mM), 0.8 µL of DMSO, 0.2 µL Taq polymerase (Invitrogen), 1.0 µL of each forward and reverse primer mentioned above and 1.0 µL template DNA. Thermal cycling conditions were 94°C for 4 min only on the first cycle, followed by 94°C for 1 min, 57°C for 1 min, 72°C for 4 min, repeated for 35 cycles with a final extension at 72°C for 10 min (Boyer *et al.*, 2001). Reactions were carried out using a thermo cycler (Eppendorf,

USA). PCR products were visualized by 1% agarose gel electrophoresis with ethidium bromide staining and UV illumination.

PCR products were diluted to 1/5 of the original concentration for sequencing reaction. Each 10 µL of reaction mixture contained 6.34 µL of sterile water, 1.0 µL of Big Dye Terminator (BDT), 1.5 µL of sequencing buffer, 0.16 µL of each primer and 1.0 µL of the diluted template. Capillary separation of Big Dye Terminator 3.1 reactions and sequencing were carried out by Landcare Research NZ Ltd., Auckland, New Zealand. Electropherograms were checked using Sequencer 4.5 (Gene Codes Corporation, Michigan, USA).

Strains of three oscillatorialean morphospecies (*Phormidium autumnale*, *P. inundatum* and *P. cf. bekesiense*) each containing the 16S-23S intergenic spacer region was cloned using TOPO TA Cloning Kit (Invitrogen) with the pCR4-TOPO vector according to the manufacturer's manual. Ten colonies of each morphospecies were selected for sequencing.

4.2.2 Sequence alignment and phylogenetic analysis

Two sets of sequence data were constructed. The first set comprised partial 16S rDNA sequences of *Placoma regulare, Heteroleibleinia fontana* and *Nostoc verrucosum, Nostoc* sp.1 and *Nostoc* sp. 2. Homologous sequences to those of the studied strains were identified using a MegaBlast search of the NCBI database in which only closely related sequences were selected to construct the phylogenetic tree. These 16S rDNA sequences were aligned using ClustalW in MEGA (version 5, Tamura *et al.*, 2011) and checked by eye. Ribosomal database project (RDP) alignment based on predicted secondary structures (Cole *et al.*, 2009) were used as the basis of alignment for ambiguous regions where possible; where not possible, these regions were deleted back to the nearest conserved base. Information on the aligned sequence for each dataset is presented in Table 4.1.

Table 4.1: Summary information for each aligned 16S rDNA sequence dataset.

Aligned sequences	Length	No. of variable sites	No. of parsimony-informative sites
Placoma regulare	1438	393	322
Heteroleibleinia fontana	1485	335	240
Nostoc verrucosum, N sp.1, *N.* sp. 2	1470	269	159

Phylogenetic analysis was carried out in MRBAYES v3.0B4 (Ronquist and Huelsenback, 2003) and PAUP*4.0b10 (Swofford, 2002). Bayesian Inference (BI) was implemented in MRBAYES. The evolutionary model used was the general-time-reversible model, GTR+G+I (Tavaré, 1986) rate matrix with six different substitution types selected in all cases using modeltest in MEGA (version 5, Tamura *et al.*, 2011). The analysis was run for 2 replicates of 8 chains each for 2.5 million generations, discarding the first 100 000-1 000 000 as burn-in based on log-likelihood plots. Examination of the

variance around the parameter estimates followed. Maximum parsimony (MP) was implemented in PAUP* and bootstrap re-sampling was performed using 1000 replications. The analysis was conducted with the following settings: branches collapsed if maximum length is 0, DELTRAN character state optimisation and assignment of character states not observed in terminal taxa allowed at internal nodes.

The second set comprised sequences of the 16S-23S intergenic spacer region of strains of *Phormidium autumnale*, *P.* cf. *bekesiense, P. inundatum, P. cf. irriguum, P. cf. subfuscum,* two strains of *P. uncinatum* and *Oscillatoria curviceps.* The closest matches to each strain were identified using a MegaBlast search and sequences were compared directly without tree building analysis. Secondary structure model predictions have been widely utilized for comparing ITS sequences (Hašler *et al.*, 2012; Iteman *et al.*, 2000; Lukešová *et al.*, 2009; Siegesmund *et al.*, 2008). This computational method determines the structures of the sequences based on hypotheses rather than observations and are rarely tested. Information such as presence and absence of tRNAs, sequence length and ease of alignment are readily obtainable by comparing straight sequences without these models.

4.2.3 Amplified fragment length polymorphism (AFLP)

Genetic diversity of *Nostoc verrucosum* collected from different streams was investigated using AFLP. Two replicate colonies were collected from each of three sites in Kaituna River (Kaituna River_Bridge, Kaituna River_Charlie and Kaituna River_Reserve), one site at Hinewai Reserve, two sites at Orton Bradley Reserve and one site at The Groynes, from moist soil on banks of Selwyn River at Whitecliffs (one site) and Glentunnel (two sites) and from irrigation ditches on Sugarloaf and along Robinson Road (Fig. 4.1).

DNA extraction, PCR and sequencing were carried out as outlined above (section 4.2.1). Partial 16S rDNA sequences for all specimens were compared to check that they were identical. Based on this, colonies collected from moist soils adjacent to Selwyn River and those from irrigation ditches were not close relatives of *N. verrucosum* and were excluded from the analysis. Colonies collected from all the remaining sites were analysed.

AFLP analysis was carried out using the method of Vos *et al.* (1995) with some modifications. The 20 µL digestion reaction mixture (6.6 µL of sterile water, 4.0 µL of T4 DNA ligase buffer (Invitrogen), 2.0 µL of EcoRI adaptor (5 µM), 2.0 µL MseI adaptor (50 µM), 2.0 µL of NaCl (0.5 M), 1.0 µL of BSA (1.0 mg/ml), 1.0 µL of EcoRI, 0.2 µL of MseI, 0.2 µL of T4 DNA ligase and 1.0 µL of DNA template) was incubated for two hours at 37˚C.

Figure 4.1: Collection sites for *Nostoc verrucosum*. Scale bar: 10km.

Pre-selective PCR was carried out for each 25 µL reaction containing 17.2 µL sterile water, 2.5 µL of 10 x buffer (Invitrogen), 1.2 µL of dNTPs (2.5 mM), 0.5 µL of each EcoRI and MseI primers, 0.1 µL of *Taq* polymerase (Invitrogen) and 3.0 µL of DNA template (diluted 1:10).

PCR conditions for amplifications were 94˚C for 120 s followed by 20 cycles of 94˚C for 30 s, 56˚C for 30 s and ˚C72 for 120 s. Selective amplifications were carried out using four primer combinations: Eco+ACT/Mse+C, Eco+ACT/Mse+CG, Eco+AC/Mse+C and Eco+A/Mse+C. These were screened for suitability for all taxa and the best primer combinations Eco+AC/Mse+C were used for the analysis.

Aliquots (5 µL) of each selective amplification product were mixed with 3 µL of formamide loading buffer (0.5 M). Mixtures were denatured at 95˚C for 5 min and cooled in ice immediately. Aliquots (5 µL) sample was loaded onto a polyacrylamide gel (on 33 x 39 cm glass plates) prepared with 48 g urea, 20 mL of 5x TBE, 15 mL of 40% acrylamide made up to 100 mL with distilled water and later mixed with 450 µL of TEMED and 20% APS. Electrophoresis was at about 100W for 2 h until the second dye front was 2/3 of the way down the gel. After electrophoresis, the gel was fixed in 2 L of 10% acetic acid for an hour. The gel was washed 4 times for 2 min each wash and stained in 2 L of water with 2 g silver nitrate and 3 ml formaldehyde for 30 min. Bands were developed in a solution of 60 g sodium carbonate and 2 L of water left chilled and later mixed with 400 µL of sodium thiosulphate and 3 mL of formaldehyde just prior to development.

Gels were scanned and the images scored using a binary scoring system based on the presence or absence of bands as 1 and 0 respectively. The data were analysed by neighbour joining analysis in Splitstree 3.2 (Huson, 1998), restML in Phylip (Felsenstein, 2003) and binary model in MRBAYES v3.0B4 (Ronquist and Huelsenback, 2003).

4.3 Results

4.3.1 Phylogenetic analysis of the 16S rDNA

A phylogenetic tree was constructed for three datasets each of: 1) *Placoma regulare* UCFM_PR (KF264594), 2) *Heteroleibleinia fontana* UCFM_HF (KF264596), and 3) *Nostoc verrucosum* UCFM_NVK (KF264592), *Nostoc* sp.1 UCFM_NGR (KF264593) and *Nostoc* sp. 2 UCFM_NV (KF264595). The trees constructed using MP and BI methods were largely congruent, therefore topologies of the BI trees are presented. Numbers associated with nodes are Bayesian posterior probability (PP)/Maximum parsimony bootstrapped (MPB) percentages. These are shown only when PP≥ 0.70 for *P. regulare* and *H. fontana* tree and PP≥ 0.50 for *N. verrucosum, Nostoc* sp.1 and *Nostoc* sp. 2 tree.

4.3.1.1 *Placoma regulare* UCFM_PR phylogeny

The analysis for the 16S rDNA gene shows that strain UCFM_PR belongs to a clade with a high support in both BI and MP trees that contains *Chamaesiphon subglobosus* PCC7430, and three uncultured cyanobacterium clones (B10805H, WB7.4 and SepB-17) (Fig. 4.2). The strain shares 97% sequence similarity with *C. subglobosus* PCC7430 over the 1084 bp 16S rDNA gene segment. *Leptolyngbya* sp. GSE PSE30 01B is the sister taxon to the clade.

4.3.1.2 *Heteroleibleinia fontana* UCFM_HF phylogeny

A 694 bp partial 16S rDNA gene segment was obtained for strain UCFM_HF. This sequence shares high sequence similarity (>98%) with two strains of *Phormidium priestleyi* (ANT.LPR2.5 and ANT.LPR2.6) and one uncultured Antarctic bacterium LB3-1 (Fig. 4.3). The uncultured Antarctic bacterium LB3-1 is most probably *P. priestleyi* appearing in an environmental clone library. Sister relationship between *H. fontana* and *P. priestleyi* is supported by MP (88%) but not by BI (0.77). *Heteroleibleinia fontana* is distant from morphologically similar *Tapinothrix* sp. GSE-PSE06-07G.

4.3.1.3 *Nostoc verrucosum* UCFM_NVK, *Nostoc* sp. 1 UCFM_NG and *Nostoc* sp. 2 UCFM_NV phylogeny

The three *Nostoc* species are not close relatives (Figs. 4.4 and 4.5). *Nostoc verrucosum* UCFM_NVK shares high sequence similarity (>95%) with *N.* cf. *verrucosum* (and *N. verrucosum* (uncultured *N. verrucosum* Ashitsuki) over the 1143 bp partial 16S rDNA sequence segment (Fig. 4.4). *Nostoc* sp. 1 UCFM_NG belongs to a clade containing strains of mutualistic *Nostoc* (Fig. 4.5) and shares high

sequence similarity (99%) with members of the clade over the 970 bp partial 16S rDNA sequence segment. *Nostoc* sp. 2 UCFM_NV lacks close relatives (Fig. 4.4).

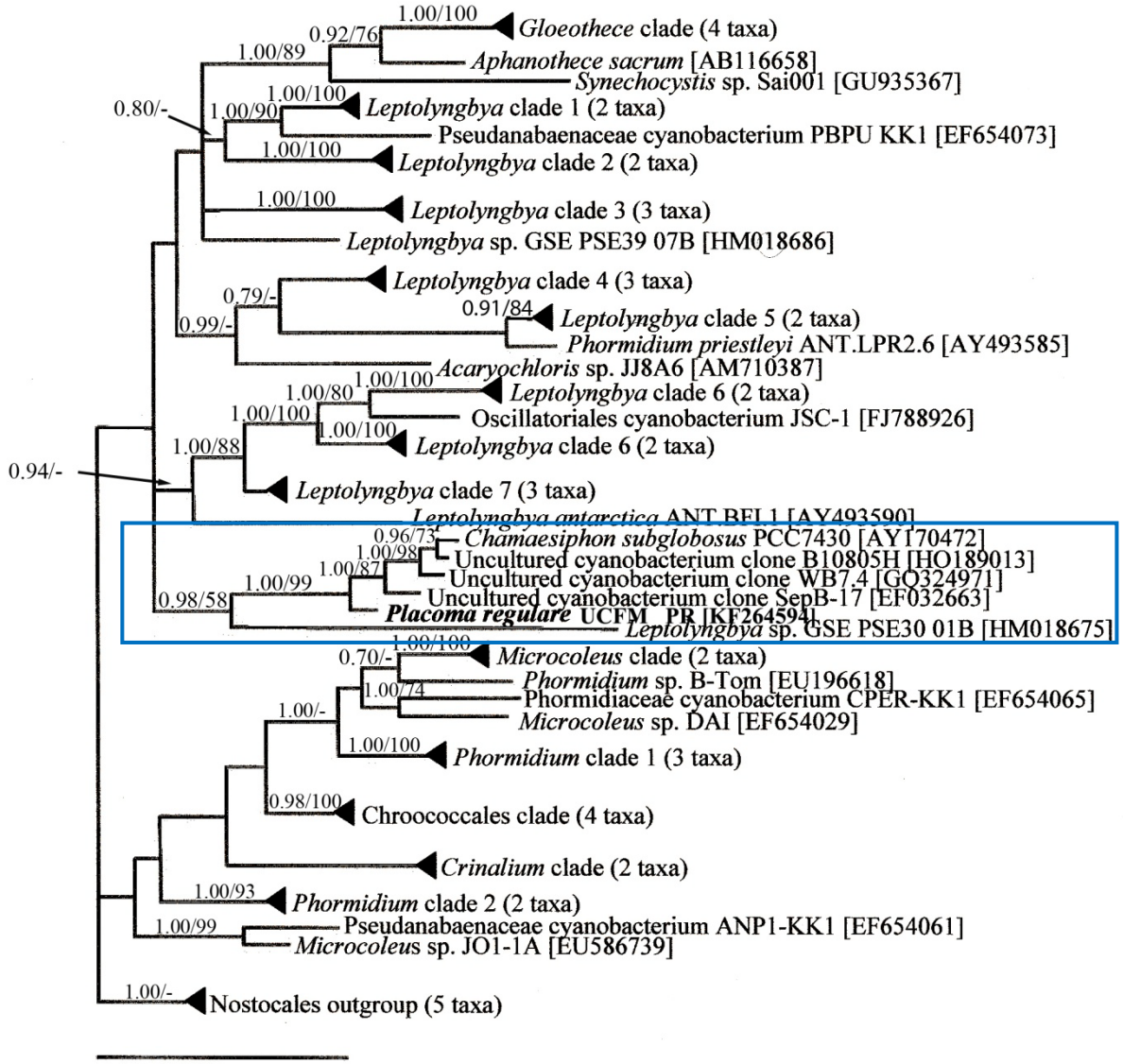

Figure 4.2: Phylogenetic analysis of *Placoma regulare* (in bold) inferred from 16S rDNA sequences. The tree topology is inferred using MRBAYES v3.0B4 (5 million generations). Numbers associated with nodes are Bayesian posterior probability (PP)/Maximum parsimony bootstrapped (MPB) percentages. These are shown only when PP $\geq$ 0.70. Part of the tree highlighted by blue box is referred to in the text. Scale bar represents 0.1 changes per site.

Figure 4.3: Phylogenetic analysis of *Heteroleibleinia fontana* (in bold) inferred from 16S rDNA sequences. The tree topology is inferred using MRBAYES v3.0B4. Numbers associated with nodes are Bayesian posterior probability (PP)/Maximum parsimony bootstrapped (MPB) percentages. These are shown only when PP $\geq$ 0.70 Parts of the tree highlighted by blue boxes are referred to in the text. Scale bar represents 0.1 changes per site.

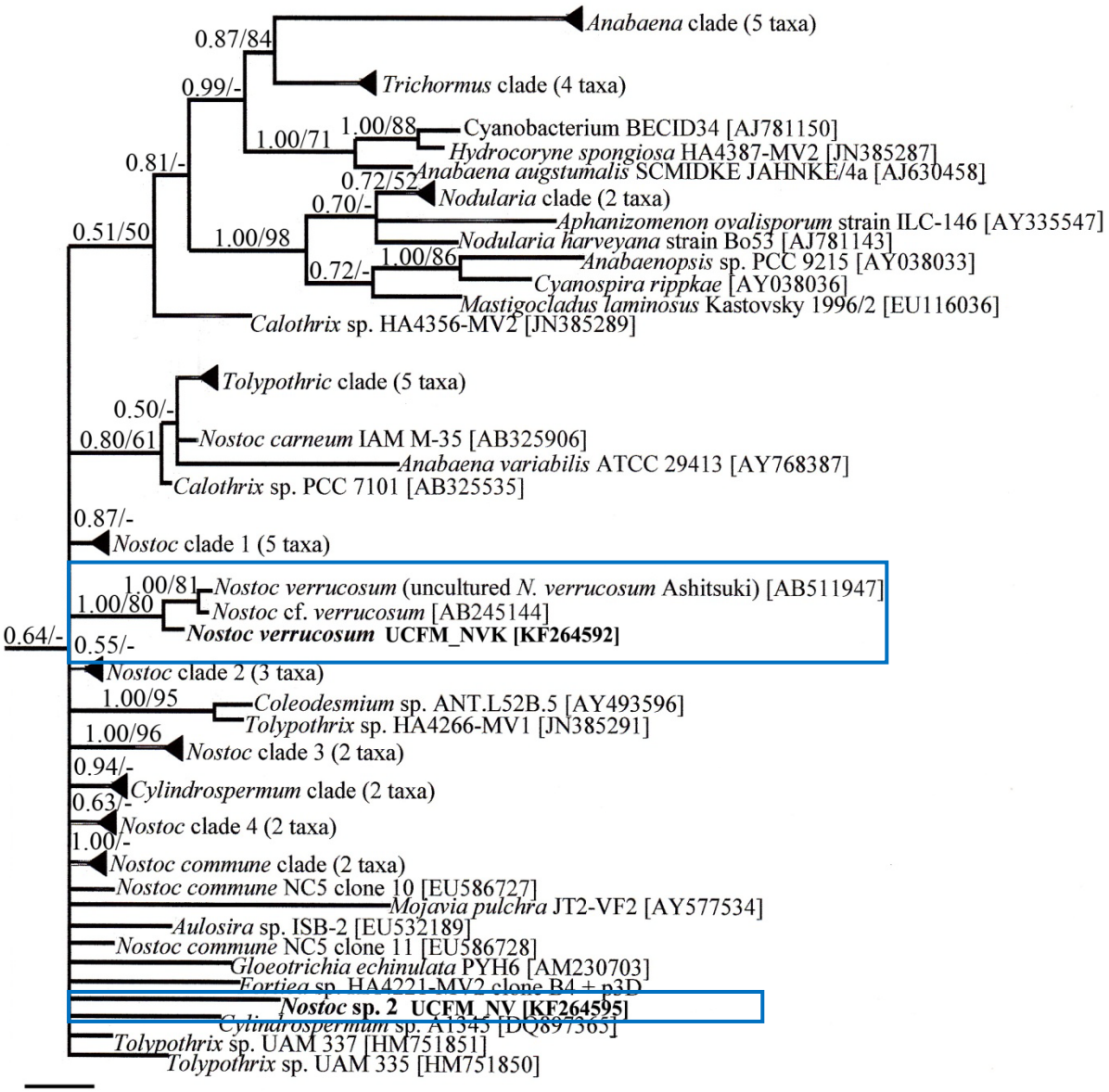

Figure 4.4: Phylogenetic tree including *Nostoc verrucosum* and *Nostoc* sp. 2 (in bold) inferred from 16S rDNA sequences. The tree topology is inferred using MRBAYES v3.0B4. Numbers associated with nodes are Bayesian posterior probability (PP)/Maximum parsimony bootstrapped (MPB) percentages. These are shown only when PP $\geq$ 0.50 Parts of the tree highlighted by blue boxes are referred to in the text. Scale bar represents 0.1 changes per site.

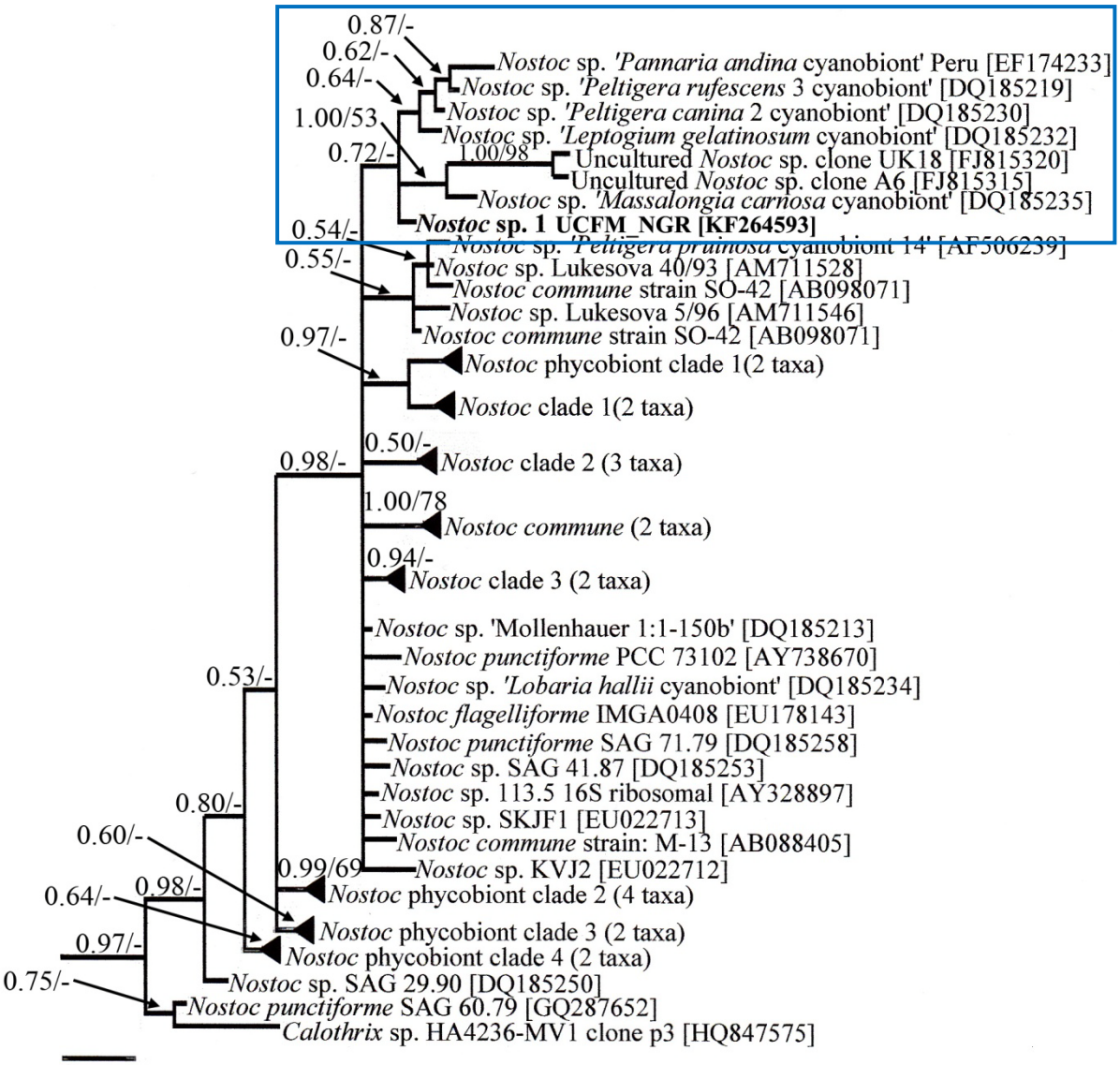

Figure 4.5: Phylogenetic tree including *Nostoc* sp. 1 (in bold) inferred from 16S rDNA sequences. The tree topology is inferred using MRBAYES v3.0B4. Numbers associated with nodes are Bayesian posterior probability (PP)/Maximum parsimony bootstrapped (MPB) percentages. These are shown only when PP≥ 0.50 The part of the tree highlighted by a blue box is referred to in the text. Scale bar represents 0.1 changes per site.

4.3.2 Analysis of the 16S-23S ITS region in eight oscillatorialean strains

A strong single band was produced from the products of amplification of the spacer region of six oscillatorialean strains: *Phormidium cf. irriguum*, *P. cf. subfuscum*, two strains of *P. cf. uncinatum*, *P. cf. bekesiense* and *Oscillatoria cf. curviceps*. Multiple bands within each of two different isolates were successfully cloned for strains of *P. autumnale* and *P. inundatum*.

The ITS sequences for six strains have two inserts, a $tRNA^{Ile}$ and $tRNA^{Ala}$ gene sequence (Table 4.2). *P.* cf. *bekesiense*, *P. inundatum* and *O.* cf. *curviceps* have only $tRNA^{Ile}$.

Table 4.2: Summary of 16S-23S intergenic spacer region of studied strains (in bold). Strains grouped by a particular shading include those with similar ITS region as identified from MegaBlast search as well as strains selected from GenBank for comparative purposes (marked by *).

Morphotypes	Accession number	D-stem	Spacer	tRNA-Ile	Spacer	tRNA-Ala	Spacer
***Phormidium autumnale* UCFM_PAS**	**KF264586**	**56**	**51**	**49**	**73**	**73**	**228**
***P.autumnale* UCFM_PA**	**KF264587**	**56**	**51**	**74**	**86**	**73**	**155**
P. autumnale VUW17	GU018029	56	51	74	86	73	121
P. autumnale CYN48	GU018024	56	51	74	86	73	121
P. autumnale CYN47	GU018023	56	51	74	86	73	121
P. autumnale VUW18	GU018028	56	51	74	86	73	121
P. autumnale CYN49	GU018025	56	51	74	86	73	121
P. autumnale VUW24	GU018031	53	51	74	87	73	123
P. autumnale VUW8	GU018021	55	51	74	85	73	116
P. autumnale CCAP1462/10	AM398959	56	51	74	84	73	116
P. autumnale LCR-OSC3	HQ012550	57	51	74	87	73	110
***Phormidium uncinatum* UCFM_PUB**	**KF264581**	**55**	**51**	**74**	**89**	**73**	**125**
P. autumnale VUW9	GU018026	55	51	74	89	73	125
***P. uncinatum*_UCFM_PUR**	**KF264582**	**60**	**51**	**74**	**87**	**73**	**100**
P. autumnale 'Hut'	EF222209	60	51	74	89	73	100
**P. uncinatum* SAG 81.79	AM398953	66	107	74	63	73	130
***Phormidium* cf. *subfuscum* UCFM_PS**	**KF264580**	**56**	**51**	**74**	**89**	**73**	**109**
P. cf. *subfuscum* I-Roc	EU196670	56	51	74	86	73	105
P. autumnale VUW11	GU018030	56	53	74	87	73	120
P. autumnale VUW2	GU018027	56	51	74	86	73	123
P. autumnale VUW4	GU018020	56	51	74	86	73	123
***Phormidium* cf. *irriguum* UCFM_PIR**	**KF264584**	**60**	**51**	**74**	**87**	**73**	**100**
**P. irriguum* f. *minor-02*	FN813339	63	44	74	25	73	131
**P.* cf. *irriguum* CCALA 759	FN813340	64	45	74	17	73	146
***Phormidium inundatum* UCFM_PIN**	**KF264583**	**66**	**50**	**74**	**256**	**-**	**-**
Synechococcus sp. PCC 7002	NC010475	65	40	74	49	73	210
**P. inundatum* SAG 79.79	AM398954	57	54	74	80	73	178
***Phormidium* cf. *bekesiense* UCFM_PB**	**KF264585**	**65**	**48**	**74**	**91**	**-**	**-**
Wilmottia murrayi KGI28	HQ873481	64	49	74	233	-	-
***Oscillatoria curviceps* UCFM_OC**	**KF264579**	**59**	**48**	**74**	**294**	**-**	**-**
Uncultured cyanobacterium clone RD069	DQ181773	59	49	74	283	-	-
Uncultured cyanobacterium clone Fr147	AF547642	59	49	74	284	-	-
**O.* cf. *curviceps* Fkv-3	EU196673	59	59	74	13	73	77
**O.* cf. *curviceps* Fkv-4	EU196674	59	59	74	13	73	77

The ITS length for most strains was in the range of 445-495 bp except for *P*. cf. *bekesiense* UCFM_PB (278 bp) and *P. autumnale* UCFM_PAS (530 bp) (Table 4.3). Seven strains analysed showed differences in the sizes of individual 16S-23S ITS region except for *P*. cf. *irriguum* UCFM_PIR which is identical to *P. uncinatum* UCFM_PUR.

Of six *P. autumnale* clones analysed, only strain UCFM_PAS had a shorter $tRNA^{Ile}$ gene sequence (Table 4.2). *P. uncinatum* UCFM_PUB showed identical ITS composition to *P. autumnale* VUW9. The sequence of a similar strain, *P. uncinatum* SAG 81.79, retrieved from GenBank, showed a significantly larger spacer region. *Phormidium* cf. *subfuscum* UCFM_PS showed similar ITS composition to *P*.cf. *subfuscum* I-Roc but had a shorter ITS length (452 bp) than the other close relatives from MegaBlast. *Phormidium* cf. *irriguum* UCFM_PIR showed identical ITS composition to *P. uncinatum* UCFM_PUR but had a significantly larger spacer compared to two *P. irriguum* sequences from GenBank (FN813339 and FN813340) (Table 4.2).

Five clones of *P. inundatum* all had one insert, $tRNA^{Ile}$. The sequence of *P. inundatum* SAG 79.79 from GenBank contained two tRNAs. *Oscillatoria curviceps* UCFM_OC showed similar ITS configuration to two uncultured cyanobacterial clones (RD069 and Fr147) but differed from sequences of *O*. cf. *curviceps* Fkv-3 and Fkv-4 from GenBank (EU196674 and EU196673).

Morphology of all the studied strains both from field specimens and cultures were analysed by light microscopy. Detailed descriptions of all eight morphospecies are presented in Chapter 3. A summary of diacritical features which includes cell lengths, cell widths and the presence of calyptra for strains used in this study (in bold) and their close relatives are presented in Table 4.3.

Cell width for both *P. autumnale* strains (UCFM_PAS and UCFM_PA) and strains from GenBank with similar ITS were mostly about 6.0 - 7.0µm wide. *Phormidium autumnale* strains VUW 17, VUW 24, VUW 8 and CCAP 1462/10 were exceptions. VUW 8 has the broadest cells ranging up to 13.2 µm. Cell lengths were variable. The majority including the study strain have isodiametric/shorter/longer than wide cells. Distinctly shorter cells are a common feature in wider filaments (VUW 17, VUW 24, VUW 8 and CCAP 1462/10). Presence of calyptra on the apical cell was a prominent feature.

The two *P. uncinatum* strains UCFM_PUB and UCFM_PUR were close relatives of *P.autumnale* VUW 9 and *P. autumnale* 'Hut'. *Phormidium autumnale* VUW 9 morphological features conform to the two *P. uncinatum* strains from the study. *Phormidium autumnale* 'Hut' and *P. uncinatum* SAG 81.79 have narrower cells, distinctly so in the latter and both lack a calyptra.

Cells of *P.* cf. *subfuscum* UCFM_PS were distinctly wider than all its close relatives (Table 4.3). Cell lengths of all the strains are generally shorter than wide. Calyptra is present in all.

P. cf. *irriguum* UCFM_PIR has distinctly wider cells than *P. uncinatum* UCFM_PUR and differs distinctly from the phenotypic features of *P. irriguum* f. *minor* ETS-02. *P. inundatum* UCFM_PIN and *P. inundatum* SAG 79.79 both have similar cell width but differ in length.

Table 4.3: Morphological characteristics of strains from the study site and its tributary stream (in bold) and their close relatives. Strains grouped by a particular shading include those with similar ITS region as identified from MegaBlast search as well as strains selected from GenBank for comparative purposes (marked by *).

Morphotypes	Accession number	**Cell width (µm)**	**Cell length (µm)**	**Calyptra**	**ITS length (bp)**
***Phormidium autumnale* UCFM_PAS**	**KF264586**	**6.0-7.0**	**2.0-5.0**	**+**	**530**
***P. autumnale* UCFM_PA**	**KF264587**	**6.0-7.0**	**2.0-5.0**	**+**	**495**
P. autumnale VUW17	GU018029	6.0-7.8	3.0-4.2	+	461
P autumnale CYN48	GU018024	6.0-7.2	3.0-6.0	+	461
P. autumnale CYN47	GU018023	6.0-7.2	3.0-6.0	+	461
P. autumnale VUW18	GU018028	6.0-7.2	2.4-3.6	+	461
P. autumnale CYN49	GU018025	5.4-6.6	2.4-4.2	+	461
P. autumnale VUW24	GU018031	6.0-9.6	3.0-4.8	+	461
P. autumnale VUW8	GU018021	9.6-13.2	1.8-4.2	+	454
P. autumnale CCAP1462/10	AM398959	5.0-9.0	<1	+	454
P. autumnale LCR-OSC3	HQ012550	5.0-6.0	3.0-7.0	+	452
***Phormidium uncinatum* UCFM_PUB**	**KF264581**	**8.0-9.0**	**2.0-6.0**	**+**	**467**
P. autumnale VUW9	GU018026	7.8-9.6	3.6-5.4	+	467
***P. uncinatum* UCFM_PUR**	**KF264582**	8.0-9.0	2.0-6.0	+	445
P. autumnale 'Hut'	EF222209	6.0-9.0	4.0-9.5	-	447
**P. uncinatum* SAG 81.79	AM398953	2.3±0.1	<1	-	513
***Phormidium* cf. *subfuscum* UCFM_PS**	**KF264580**	**10.0-12.5**	**2.0-5.0**	**+**	**452**
P. cf. *subfuscum* I-Roc	EU196670	6.9-7.8	3.5-4.0	+	465
P. autumnale VUW11	GU018030	6.0-7.2	2.4-4.8	+	463
P. autumnale VUW2	GU018027	6.6-7.8	2.4-3.6	+	463
P. autumnale VUW4	GU018020	7.2-7.8	4.2-6.6	+	463
***Phormidium* cf. *irriguum* UCFM_PIR**	**KF264584**	**11.0-12.0**	**2.5-8.0**	**+**	**445**
**P. irriguum* f. *minor* ETS-02	FN813339	3.0-5.0	1.0-2.0	-	410
**P.* cf. *irriguum* CCALA 759	FN813340	9.0-12.0	3.0-5.0	+	419
***Phormidium inundatum* UCFM_PIN**	**KF264583**	**4.0-5.0**	**5.0-7.0**	**-**	**446**
**P. inundatum* SAG 79.79	AM398954	4.1-5.0	<1	-	516
***Phormidium* cf. *bekesiense* UCFM_PB**	**KF264585**	**9.0-10.0**	**6.0-10.0**	**-**	**278**
Wilmottia murrayi KGI28	HQ873481	3.6-4.5	3.4-7.2	-	420
***Oscillatoria curviceps* UCFM_OC**	**KF264579**	**10.0-12.0**	**1.2-3.8**	**-**	**475**
**O.* cf. *curviceps* Fkv-3	EU196673	13.2-15.1	6.0-7.0	-	355
**O.* cf. *curviceps* Fkv-4	EU196674	13.2-15.1	6.0-7.0	-	355

Wilmottia murrayi KGI28 appears to be a close relative of *P.* cf. *bekesiense* UCFM_PB but the two did not conform morphologically. No sequence data was available for other *P.* cf. *bekesiense* in GenBank.

Oscillatoria curviceps UCFM_OC showed no morphological resemblance to *O.* cf. *curviceps* Fkv-4 and Fkv-3.

4.3.3 AFLP analysis of field specimens of *Nostoc verrucosum*

Primer pair Eco+AC/Mse+C generated 32 polymorphic bands and gave the best signal. All three different analysis methods produced the same main splits separating Kaituna and Hinewai specimens from Orton Bradley and Groynes specimens. Most of the replicate samples are also grouped together. Support values for the main splits (Fig. 4.6) from the three analyses are presented in Table 4.4. Despite the low values, all three methods showed a consistent result. The three phylogenetic trees were largely congruent; therefore topology of the neighbour joining analysis is presented here (Figure 4.6).

Table 4.4: Support values for main splits obtained from different analysis. Neighbour joining and restML values are bootstrapped percentages while values for binary model are Bayesian posterior probability. See Fig. 4.6 for positions of splits.

	Support value for each main split		
Methods employed	1	2	3
Neighbour joining (Splitstree 3.2)	78	49	34
restML (Phylip)	50	38	48
Binary Model (MRBAYES)	0.81	0.67	0.88

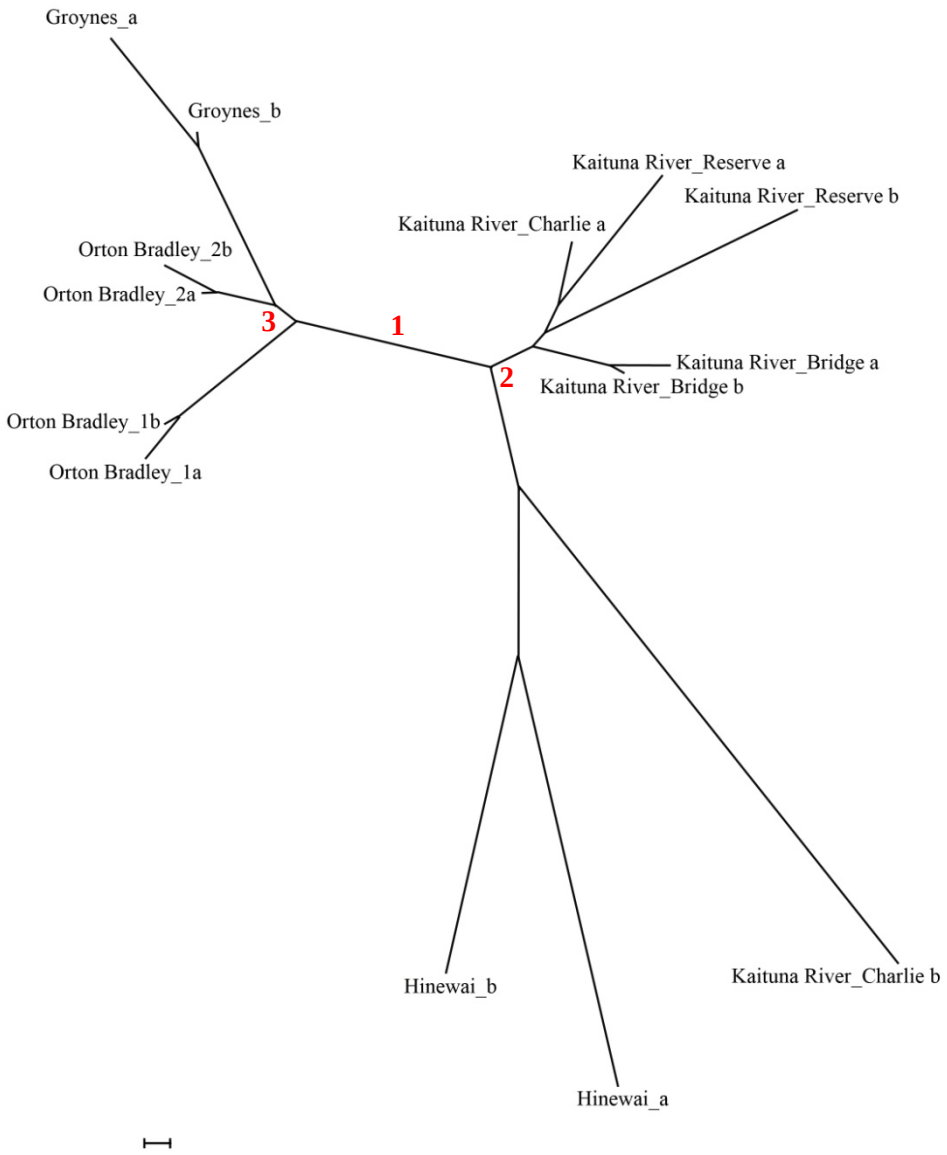

Figure 4.6: Unrooted phylogenetic tree for *Nostoc verrucosum* collected from four catchments inferred by neighbour joining analysis of AFLP data. The main splits are labelled in red. Scale bar represents 0.01 changes per site.

4.4 Discussion

4.4.1 16S rDNA phylogeny

4.4.1.1 *Placoma regulare*

Placoma regulare is a colony forming chroococcalean that belongs to the family Hydrococcaceae Kützing. The genus is poorly known worldwide (Komárek and Anagnostidis, 1999). Only seven species have been recorded of which three are from freshwater (Broady and Ingerfeld, 1991; Skuja, 1949; Wille, 1903). Descriptions of the Kaituna field specimens and cultured strain are presented in Chapter 3. The morphospecies *P. regulare* was erected from specimens from South Island, New Zealand streams by Broady and Ingerfeld (1991).

The phylogenetic clustering of *Placoma regulare* UCFM_PR with *Chamaesiphon subglobosus* PCC7430 and three uncultured cyanobacteria clones (B10805H, WB7.4 and SepB-17) complies with the 95% threshold for a congeneric bacterial genus as suggested by Stackebrandt and Goebel (1994). The close affiliation of *P. regular*e UCFM_PR and *C. subglobosus* PCC7430 is interesting as traditional taxonomy based on morphology separates them into different families. *Chamaesiphon subglobosus* is a member of the Chamaesiphonaceae Borzì (1882). The main characteristic feature of the family is the ability to form exospores as a product of asymmetric cell division of heteropolar mature cells. This is a distinctive feature compared to the Hydrococcaceae in which cells divide in various planes and reproduction is by release of single or small clusters of cells from the periphery of colonies. Exospores are not formed (Komárek and Anagnostidis, 1999).

Morphological attributes clearly separate the two morphospecies but molecular data suggest that they are close relatives. A similar situation has been reported for non-spore forming and spore-forming unicellular strains of the order Pleurocapsales (Ishida *et al.*, 2001). Both morphotypes were originally thought to constitute a single clade (Honda *et al.*, 1999; Nelissen *et al.*, 1994). However, as the number of pleurocapsalean strains included in the 16S rDNA analysis increased, it became clear that each morphotype constituted a separate clade (Ishida *et al.*, 2001). The phylogeny presented here is inconsistent with present use of exospore formation as a diagnostic character to differentiate the Chamaesiphonaceae from the Hydrococcaceae.

Placoma regulare UCFM_PR, *C. subglobosus* PCC 7430, uncultured cyanobacterium clones WB7.4 and SepB-17 all originated from streams. In contrast, uncultured cyanobacterium clone B10805H was from soil below snow from the Annapurna Range, Nepal (Schmidt *et al.*, 2011). Clone B10805H is a close relative of stream algae.

4.4.1.2 *Heteroleibleinia fontana*

Heteroleibleinia fontana is a member of the Pseudanabaenaceae Anagnostidis and Komárek 1988, subfamily Heteroleibleiniodeae Anagnostidis et Komárek 1997. This is another poorly known genus that has not previously been included in molecular phylogenies (Komárek and Anagnostidis, 2005).

Heteroleibleinia fontana UCFM_HF, two strains of *Phormidium priestleyi* and an unidentified Antarctic bacterium form a clade supported by MP but not by BI. *P. priestleyi* is a member of the Phormidiaceae Anagnostidis et Komárek 1998, subfamily Phormidiodeae Anagnostidis et Komárek 1986. The trichome morphology of *P. priestleyi* closely resembles that of *H. fontana* except that trichomes of *P. priestleyi* are isopolar. *P. priestleyi* forms mats on rocks in streams in maritime Antarctica (Fritsch, 1917).

Although heteropolarity of *H. fontana* is an important taxonomic character (Komarek and Anagnostidis, 2005), this feature is unstable. In unialgal cultures, the filaments become isopolar and do not exhibit basal attachment to agar surfaces (Chapter 3). However, in mixed species enrichment cultures where a suitable surface was available for attachment, e.g. empty sheaths of *Chamaesiphon*, colony formation resembled that of populations in nature.

Descriptions of the morphology of *P. priestleyi* ANT.LPR 2.5 and ANT.LPR 2.6 have been made from clonal unialgal cultures established from mats collected from lakes in Antarctica (Taton *et al.*, 2006). The filament morphology conforms to the type description of *P. priestleyi* by Fritsch (1917) apart from the slightly narrower trichome width and the lake rather than stream habitat. However, in the phylogenetic tree, these two strains occupied a position distant from typical morphospecies of *Phormidium*. A new genus *Phormidesmis* has been erected (Turicchia *et al.*, 2009) based on *Phormidium molle* from tropical alkaline marshes. This is distant from typical *Phormidium* morphospecies and classified as a separate cluster in Pseudanabaenaceae in 16S rDNA phylogenies (Komárek *et al.*, 2009). According to Komárek *et al.* (2009), *P. priestleyi* is very similar to *P. molle* in all morphological and cytological characteristics and must be assigned to the vicinity of pseudanabaenacean genera according to molecular evaluation. Hence, the tentative relationship between *P. priestleyi* and *H. fontana* in the present analysis further supports the authors findings by placing the two in the same family.

A sequence for *Tapinothrix* sp. GSE-PSE06-07G from GenBank was included in the analysis as it belongs to the same subfamily as *Heteroleibleinia*. *Tapinothrix* is defined by the ability of the apical cell to elongate into a fine hair (Komárek and Anagnostidis, 2005). Although the two genera are separated only by this single morphological character, phylogenetic analysis places them in well-separated clusters (Figure 4.3). The closest relative to *Tapinothrix* sp. was a *Leptolyngbya*, also a member of the family Pseudanabaenaceae. Morphological separation of the two morphotypes seems

slight although molecular information suggest that Heteroleibleiniodeae as currently circumscribed is polyphyletic and should be revised.

4.4.1.3 *Nostoc verrucosum*, *Nostoc* sp. 1 and *Nostoc* sp. 2

Nostoc Vaucher ex Bornet et Flahault 1888 belongs to the Nostocales and Nostocaceae according to traditional taxonomy (Komárek and Anagnostidis, 1989). It is widespread (Dodds *et al.*, 1995) in freshwater benthos and plankton (Hoffmann, 1996; Mollenhauer *et al.*, 1999) while also forming mutualisms with plants and fungi (Guevara *et al.*, 2002). Identification of the morphospecies is based primarily on colony structure and secondarily on trichome, vegetative cell, akinete and heterocyte shape and dimensions (Komárek and Anagnostidis, 1989).

The extreme morphological flexibility, influenced by environmental conditions and life-cycle stage, has resulted in uncertainty in identification of morphospecies based on the traditional approach (Mollenhauer *et al.*, 1994; Dodds *et al.*, 1995). To resolve this, supplementary genetic information should be included for a reliable classification of species within the genus (Rasmussen and Svenning, 2001). Recent phylogenetic studies with several marker genes including 16S rDNA, *rpo*B, *rbc*LX and *nif*H or *nif*D revealed a close relationship of *Nostoc* to *Anabaena*, *Aphanizomenon* and *Trichormus* which are all members of the Nostocaceae (Henson *et al.*, 2004; Rajaniemi *et al.*, 2005; Svenning *et al.*, 2005). According to Rajaniemi *et al.* (2005), certain morphological features of the group are stable and could be used to separate different phylogenetic clusters.

The three *Nostoc* morphospecies analysed in this study were phylogenetically distinct. Detailed descriptions of field populations are given in Chapter 3. *Nostoc verrucosum* UCFM_NVK belongs to a well-supported clade in both BI and MP trees that contains *N. cf. verrucosum* (AB245144) and uncultured *N. verrucosum* (Ashitsuki) all of which originated from streams. The present molecular phylogeny supports *N. verrucosum* as a valid distinct morphospecies.

Colonies of *Nostoc* sp. 1 do not resemble those of any other freshwater species described in the available literature. Detailed description of the field population is given in Chapter 3. Phylogenetic clustering of *Nostoc* sp. 1 UCFM_NGR with strains of *Nostoc* that are lichen phycobionts was unexpected as it was a free-living form and showed no sign of being lichenized. The phycobionts were isolated from lichens of the genera *Massalongia*, *Peltigera* and *Pannaria* (O'Brien *et al.*, 2005; Elvebakk *et al.*, 2008). These genera have been recorded from Banks Peninsula (Galloway, 2007). This suggests two possibilities, 1) phycobionts become free-living in streams when the environment is suitable or 2) lichen fungi have the ability to recruit partners from propagules dispersed out of the stream. Sister relationships between free-living *Nostoc* and phycobionts have been demonstrated previously (Papaefthimiou *et al.*, 2008). It would be useful to compare the relationships of *Nostoc* phycobionts isolated from Banks Peninsula lichens with the isolate from the study site.

Nostoc sp. 2 UCFM_NV also had colony morphology distinct from other previously described freshwater species so far as could be ascertained. Detailed descriptions of the field population and cultures are presented in Chapter 3. Within the 16S rDNA gene phylogeny it occupied an isolated position outside all the *Nostoc* clades. Further work involving phenotypic and genotypic characterizations is required to gather more information on the relationships of *Nostoc* sp. 2 UCFM_NV.

4.4.2 16S-23S ITS analysis for eight oscillatorialean strains

Previous studies on cyanobacterial intergeneric transcribed spacer regions used fragments that varied in size from 354-694 nucleotides (Iteman *et al.*, 2000; Boyer *et al.*, 2001; Otsuka *et al.*, 2001; Palinska and Marquardt, 2007). The seven strains of *Phormidium* and one strain of *Oscillatoria* examined in this study had ITS regions ranging in length from 278-530 nucleotides.

Four out of five *P. autumnale* strains showed almost identical ITS configuration with five representatives of *P. autumnale* from GenBank apart from the larger spacer region. *Phormidium autumnale* UCFM_PAS is an exception with a 25 bp shorter $tRNA^{Ile}$. This difference in strain UCFM_PAS is remarkable given the fact that tRNAs are among the most conserved of molecules due to their vital role in the translation of nucleic acid messages into amino acids of proteins (Li-Yeh *et al.*, 2010). $tRNA^{Ile}$ and $tRNA^{Ala}$ have been proven to be identical in nucleotide sequence with variable regions identified outside the sequences of the two genes (Purcell *et al.*, 1999; Chen *et al.*, 2000). Presence or absence of the two tRNA sequences between multiple copies of the ITS region within some cyanobacterial genomes have been reported previously (Boyer *et al.*, 2001; Iteman *et al.*, 2000; Marquardt and Palinska, 2007; Sciuto *et al.*, 2012). However, in all cases, if tRNAs were present then they were highly conserved. The short tRNA sequence in the single *P. autumnale* strain suggests that it might not have retained its function. An interesting test would be to determine the genetic code for isoleucine in strain UCFM_PAS but such work is outside of the scope of the present study.

Morphology of all the strains was compared with the description of the morphospecies as given by Komárek & Anagnostidis (2005). Six strains identified as *P. autumnale* in GenBank conformed in trichome width to the definition of the species given by Komárek & Anagnostidis (2005). Three strains, VUW 24, VUW 8 and CCAP 1462/10, have cells that at the maximum of their width range exceed the maximum of 7.0 µm for *P. autumnale*. The most striking difference in morphology was observed in strain VUW 8. This was originally identified as a morphospecies of *Oscillatoria* based on the wide trichomes (9.6-13.2 µm) with cells distinctly shorter than wide and a hemispherical calyptra (Heath *et al.*, 2010). However, the 16S rDNA analysis of strain VUW 8 with two other morphologically similar strains placed these in their own clade within the *P. autumnale* lineage (Heath *et al.*, 2010). The present ITS analysis supports this finding as strain VUW 8 displays almost identical ITS configuration to a narrower *P. autumnale* strain LCR-OSC3.

Variations in cell size have been documented amongst strains of *P. autumnale* (Palinska and Marquardt, 2008) but these are less than the variation observed in strain VUW 8. Despite all the phenotypic differences, the different molecular markers showed that strain VUW 8 belongs to *P. autumnale*.

Two strains of *P. uncinatum* (UCFM_PUB and UCFM_PUR) were a close match to two strains of *P. autumnale* (VUW 9 and *P.autumnale* 'Hut'). *Phormidium uncinatum* UCFM_PUB, which was isolated from a dark brown leathery mat, showed identical ITS configuration to *P. autumnale* VUW 9. *Phormidium uncinatum* UCFM_PUR, which originated from a reddish brown leathery mat had an almost identical configuration to *P. autumnale* 'Hut'. Both *P. autumnale* strains have trichome widths that conform more closely to those of the wider *P. uncinatum* (Komárek & Anagnostidis, 2005) and perhaps would be better assigned to that morphospecies. However, *P. autumnale* 'Hut' did not possess a calyptra which contradicts the descriptions of Komárek & Anagnostidis (2005) for both *P. uncinatum* and *P.autumnale* morphospecies. Despite this, the 16S rDNA of both strains showed clustering with *P. autumnale* representatives from GenBank (Wood *et al.,* 2007; Heath *et al.,* 2010).

Uncertainty with the classification of these two morphospecies is evident within the classical approach. Komárek & Anagnostidis (2005) separate the two morphospecies based on cell length and cell shape. *Phormidium autumnale* is placed in their Group VII on the basis of the gradual or abrupt tapering of the trichome, isodiametric or longer cells and the presence of a calyptra. *Phormidium uncinatum* is placed in their Group VIII based on abrupt tapering of the cylindrical trichomes, distinctly shorter cells and the presence of a calyptra. Whitton (2011) combined the two morphospecies because of the overlapping range in cell width (*P. autumnale* 4-7 µm and *P. uncinatum* 5.5-9.0 µm). The ITS sequences of our *P. uncinatum* and *P. autumnale* strains are different and this supports their identification as two different morphospecies (Chapter 3). The two *P. autumnale* sequences from GenBank that are close relatives of the *P. uncinatum* strains of this study might be better placed in the morphospecies *P. uncinatum* but the lack of a calyptra in *P. autumnale* 'Hut' remains a difficulty. Komárek & Anagnostidis (2005) stressed the importance of examining plentiful material to confirm the presence of a calyptra in well-developed mature trichomes otherwise this structure can easily be overlooked.

Phormidium uncinatum SAG 81.79 from GenBank showed a different ITS configuration to both *P. uncinatum* strains UCFM_PUB and UCFM_PUR (Table 4.3). It also has morphological differences, these being distinctly narrower and shorter cells with the absence of a calyptra. This suggests that either SAG 81.79 is misassigned to this morphospecies or that genetic changes during maintenance in culture have resulted in altered morphology (Palinska *et al.,* 1996).

Phormidium cf. *subfuscum* UCFM_PS showed almost identical ITS configuration to *P.* cf. *subfuscum* I-Roc from GenBank although the two strains differ in cell width (Table 4.3). Three *P. autumnale*

strains (VUW 11, VUW 2, and VUW 4) are the closest relatives to strain UCFM_PS from BLAST search of the studied ITS sequence. Two of these (VUW 11, VUW 2) conform closely to the morphology of *P.* cf. *subfuscum* I-Roc. VUW 4 has longer cells across its whole range of cell length. The ITS sequences place these morphospecies close together so their separation into two groups (*P. subfuscum*, Group VIII; *P. autumnale*, Group VII of Komárek & Anagnostidis (2005) suggests that morphological differences used to define these groups do not reflect the molecular data.

Both *P.* cf. *irriguum* UCFM_PIR and *P. uncinatum* UCFM_PUR from the study site had identical ITS structure although the former showed distinctly wider cells. Cell width for *P. uncinatum* is maximally up to 9.5 µm wide while *P. irriguum* has cells 6.0-11.2 µm wide (Komárek & Anagnostidis, 2005). The original identification of the field material was based on this characteristic hence separating the specimens into two distinct morphospecies. Strain UCFM_PIR had a different ITS structure from *P. irriguum* f. *minor* ETS-02P and *P. cf. irriguum* CCALA 759. This result coincides with the difference observed in the morphology of the study site specimens from these other two strains. Although *P. cf. irriguum* CCALA 759 and *P. irriguum* f. *minor* ETS-02P differ greatly in cell width, their ITS configurations are similar. The taxonomy of this morphospecies requires further study.

The ITS sequence of *P. inundatum* UCFM_PIN differed from that of *P. inundatum* SAG 79.79 (Table 4.2). They also differ in morphology in that strain UCFM_PIN has considerably longer cells (Table 4.3).

Oscillatoria curviceps UCFM_OC showed similar ITS structure to two uncultured clones of cyanobacteria (Fr147 and RD069) that originated from benthic microbial mats in Antarctic lakes (Taton *et al.*, 2003; 2006). No morphological data were available for comparison. Similar strains from GenBank, *O.* cf. *curviceps* Fkv-3 and Fkv-4, showed different ITS structures and morphological features to strain UCFM_OC. *Oscillatoria* cf. *curviceps* Fkv-3 and Fkv-4 both have longer and wider cells compared to the study strain. The cell widths for both strains fall within the given range for *O. curviceps* (Komárek & Anagnostidis, 2005) but cell length exceeded the range. Both strains Fkv-3 and Fkv-4 also differed from *O. curviceps* in trichome pigmentation and constriction at cross walls (Lokmer, 2007) but there was a lack of detailed examination of both strains which makes the comparison (i.e. use of cf.) with *O. curviceps* doubtful. Their original habitat was also very different as they were collected from flowerpots in a tropical greenhouse.

All these results indicate the need for a revision of the diacritical morphological features used to identify morphospecies of *Phormidium*.

4.4.3. Amplified fragment length polymorphism (AFLP).

All three trees were largely congruent despite the low support value. The posterior probabilities in the tree from the Bayesian analysis are higher than the bootstrap support values for the corresponding branches in the other two analyses. Variations in the values are probably due to the different characteristics of the data being measured and assumptions made by the different programs. Despite this, all the main splits were consistent throughout the three analyses. The generated trees show that the dominant dispersal event in these populations is local dispersal within each catchment. Cross catchment dispersal is infrequent (Fig. 4.6).

All the catchments sampled in this study are relatively close, separated only by approximately 11 km (Kaituna and Orton Bradley) to 52 kilometers (Hinewai and Groynes). The two immediately adjacent catchments (Kaituna and Orton Bradley) appear to have the most dissimilar genotypes. Based on the proximity of the catchments, the probable ease of dispersal of *Nostoc* propagules should enhance cross catchment dispersal. *Nostoc* can be dispersed in several ways. Akinetes are thick-walled resting cells that probably assist in long range airborne dispersal (Bhagawan and Pande, 1988, Kaplan-Levy *et al.*, 2010) and transport by birds (Padisák, 1998). They germinate on the return of suitable conditions for vegetative growth (Kaplan-Levy *et al.*, 2010). It would have been expected that cross catchment dispersal by windblown akinetes and dried fragment of colonies would be likely to occur frequently. The results, however, suggested otherwise.

Cross catchment dispersal may be successful under favourable conditions and depends on the availability of dispersal vectors and suitable niches for colonisation. The present result indicates possible barriers to dispersal between the catchments but it is difficult to suggest what these barriers might be. Environmental factors including light availability, water temperature and nutrient content (nitrogen and phosphorus) have been identified as factors influencing akinete germination (Kaplan-Levy *et al.*, 2010). This suggests that although akinetes can be widely dispersed, *in-situ* environmental conditions would determine the germination and establishment of new colonies. There could also be selection for particular genotypes in particular catchments if environmental conditions between catchments differed. These aspects require further investigation.

The ability of some strains of *Nostoc* to withstand desiccation could enhance cross catchment dispersal by windblown dried colony fragments. Tolerance to extreme desiccation of vegetative cells of air-dried colonies of the terrestrial *Nostoc commune* has been demonstrated by Potts (1999). Vegetative cells of *N. commune* were able to maintain their viability despite several decades of storage at ambient temperatures. Colonies of aquatic *N. verrucosum* may also be subjected to periodic drying when exposed to the air during periods of low flow. It could be tolerant to desiccation. However, Sakamoto *et al.* (2011) found it was sensitive to desiccation compared to *N. commune*.

They found that the massive extracellular matrix produced by colonies of *N. verrucosum* is not always linked to extreme desiccation tolerance.

To achieve high accuracy in AFLP based studies, Krauss (2000) found that most procedures for estimating diversity yielded better results when about 30 individuals were analysed per population. As our sample size is smaller than this recommendation, interpretation of the results should proceed with caution. A more intensive collection of *N.verrucosum* from more than three of the > 30 catchments on Banks Peninsula would greatly improve the power of the study.

Chapter 5

Spatial and temporal patterns in the distribution of cyanobacteria

5.1 Introduction

5.1.1 Cyanobacteria periphyton in flowing waters

Cyanobacteria are very common and widely distributed member of stream periphyton communities. They are the second most dominant component in many stream studies (Sheath and Cole, 1992; Sheath *et al.*, 1996; Branco and Necchi Jr, 1996; Lindstrøm *et al.*, 2004; Sherwood, 2006; Yang *et al.*, 2009; Krupek and Branco, 2012) and dominant in others (Sheath and Cole, 1996; Sheath and Müller, 1997; Filkin *et al.*, 2003; Krupek and Branco, 2012). Microscopic cyanobacteria co-occur with diatoms, bacteria and fungi forming communities of diverse microorganisms termed periphyton (Stevenson *et al.*, 1996). Periphyton are attached to or associated with various substrata. Epilithon refers to communities growing on rock surfaces while epiphyton and epipelon are terms used for communities growing on larger submerged plants and algae and on sediments respectively (Stevenson *et al.*, 1996).

Stream macroalgae can be defined as benthic morphospecies occurring in flowing freshwater with distinct growth forms recognizable with the naked eye (Sheath and Cole 1992). Many of the periphytic cyanobacteria are present as macroalgae in the form of mats, gelatinous colonies, tufts and conspicuous crust. Mats are the most commonly noted macroscopic form (Sheath and Cole, 1992; Wynn-Williams and Edwards, 2000; Branco *et al.*, 2001; Bonilla *et al.*, 2005; Vis *et al.*, 2008; Krupek and Branco, 2012). Cyanobacterial mats can be relatively dynamic on an areal basis in contrast with other macroscopic growth forms (Stuck and Ward, 1991). Each macroscopic growth form has various adaptive features to withstand flow related stress (Biggs *et al.*, 1998).

Periphyton serves as a primary source of energy in aquatic food webs in many streams and rivers (Minshall, 1978; Vannote *et al.*, 1980; Stevenson *et al.*, 1996), even in heavily shaded ones (Mayer and Likens, 1987). Their abundance are influenced by interrelated environmental factors that vary spatially and temporally, including light (DeNicola *et al.*, 1992; Ensminger *et al.*, 2005; Fanta *et al.*, 2010), current (Blenkinsopp and Lock, 1994; Hart and Finelli, 1999; Passy, 2001), substrata characteristics (Sabater *et al.*, 1998; Murdock and Dodds, 2007), water quality, (Veraart *et al.*, 2008; Kopp *et al.*, 2012; Loza *et al.*, 2013a) and grazing pressure (Steinman *et al.*, 1990; Wellnitz and Leroy Poff, 2006). Physical disturbance during spates resulting in abrasion and scouring of the substratum

can cause significant decline in periphyton biomass (Stevenson *et al.*, 1996; Yang *et al.*, 2009) and changes the community composition with less vulnerable prostrate species dominating spate-prone streams (Peterson and Stevenson, 1992; Biggs and Thomsen, 1995;Yang *et al.*, 2009).

5.1.2 Periphyton in New Zealand rivers and streams

The relationship between periphyton community and environmental variables has been the focus of many excellent detailed studies (Biggs, 1990; Biggs and Gerbeaux, 1993; Biggs and Smith, 2002; Quinn *et al.*, 1997b; Suren *et al.*, 2003). However, the ecology of cyanobacteria in periphyton has not been well-documented. In an extensive survey of periphyton and habitat conditions at 101 sites in rivers throughout New Zealand during summer low flow, Biggs (1990) recorded the dominance of filamentous algae taxa. These consisted mainly of green algae and diatoms. He found 22 habitat variables varying significantly across the community groups indicating strong habitat associations with periphyton communities. Conductivity was the variable found most strongly associated with community types and was suggested to be a useful indicator for classifying river habitats.

Effect of land-use on stream habitat, water quality and periphyton biomass have been described by Quinn *et al.* (1997b) from a survey of 11 hill-country streams. The study reported marked differences between periphyton biomass in streams flowing through pasture compared with those flowing through native vegetation. There was a correlation with the degree of shading with greater biomass in more exposed streams that flowed through pasture. This was also demonstrated by Quinn *et al.* (1997a). In two gravel-bed rivers, Biggs and Gerbeaux (1993) found a positive correlation between periphyton biomass and conductivity. Pulses of high water flow flushed out much of the periphyton and re-set the system for renewed growth. The study also listed the common periphyton taxa as diatoms and green algae.

The relationships between development of periphyton and flow have been thoroughly investigated. Biggs and Thomsen (1995) conducted an experimental study in a laboratory flow tank to examine the resistance of diatoms and green algae against spates. The effect of disturbance by spates varied depending on the initial taxonomic composition of the resident communities. Variations in velocity have been demonstrated to control periphyton biomass (Biggs *et al.*, 1998). Within a single reach in which there was spatial variation in flow velocity, faster flows supported the growth of coherent mucilaginous diatoms community whilst slower flows supported long filamentous green algal community. Stress effects of flooding have been reported to reduce algal biomass and alter community composition in a third order gravel-bed stream in New Zealand (Francoeur *et al.*, 1998). The algal community in this study was dominated by cyanobacteria and diatoms. *Amphitrix* (possibly a misidentification of *Homoeothrix*) and *Lyngbya* were the only cyanobacteria represented. Suren *et al.* (2003) examined the effects of low flows on periphyton in two Canterbury rivers with contrasting nutrient-enrichment. They found a succession in dominant taxa in the enriched system from diatoms

and cyanobacteria to filamentous green algae over summer. *Phormidium* was the only periphytic cyanobacteria identified.

5.1.3 Periphytic cyanobacteria as indicators of stream water quality

Rapid responses of periphyton to environmental change are useful for monitoring water quality, especially due to the pervasive anthropogenic influence of additional inorganic nutrient. Large inputs of phosphorus and nitrogen can result in periphyton growth that alters lotic community structure and ecosystem function (Smith *et al.*, 1999; Dodds, 2007; Hill *et al.*, 2010). Numerous studies have shown the value of using diatoms for monitoring stream (Bere and Tundisi, 2010; Blanco and Becarés, 2010; Delgado *et al.*, 2010; Fisher *et al.*, 2010; Stevenson *et al.*, 2010). Many others (Lindstrøm *et al.*, 2004; Porter *et al.*, 2008; Schneider and Lindstrøm, 2009; Stancheva *et al.*, 2012) have shown a relationship between non-diatom periphyton, including cyanobacteria, with different environmental variables particularly nutrient concentration, suggesting their usefulness in monitoring stream water quality.

Relationships between periphytic cyanobacteria community and changes in nutrient concentrations in streams have been reported. Mulholland *et al.* (1995) found the percentage biovolume of cyanobacteria, increased with distance downstream together with decrease in nitrate and phosphate concentrations. Conversely, Perona *et al.* (1998) and Douterelo *et al.* (2004) found the diversity of epilithic cyanobacteria from Spanish rivers, decreased with increasing eutrophication downstream.

Changes have also been demonstrated in composition of the cyanobacterial community in relation to water quality. Increases in abundance of heterocyteous cyanobacteria have been observed in response to low nitrate concentrations (Porter *et al.*, 2008; Stancheva *et al.*, 2012). Heterocyteous genera, including *Calothrix*, *Scytonema*, *Nostoc* and *Rivularia*, have been associated with low nutrient concentrations (Biggs, 2000b; Douterelo *et al.*, 2004). Conversely, mass growth of species of Oscillatoriales may be associated with eutrophication (Yu *et al.*, 1995; Vis *et al.*, 2008).

Morphological features may also provide valuable information on the nutrient status of a site. Presence of well-developed multicellular hyaline hairs in many filamentous forms is a response to phosphorus limitation (Whitton, 2008; 2011). Higher numbers of heterocytes in trichomes are indicative of water lacking combined nitrogen (concentration of nitrate and/or ammonium) in comparison to other nutrient, especially phosphate (Whitton, 2011). Presence of polyphosphate granules is indicative of a phosphorus-rich environment (Gibson and Whitton, 1987). These can be seen using light microscopy.

5.1.3 Aims

The following aspects of the ecology of macroscopic growths of periphytic cyanobacteria have been investigated.

1. Spatial and temporal patterns in their abundance and taxonomic composition along the whole study site in relation to environmental variables.
2. Small-scale changes in their abundance and taxonomic composition at nine selected locations within the study site in relation to the resistance of different growth forms to variation in flow, as well as recolonization rates and successions in dominant taxa following spates.

5.2 Methods

5.2.1 Sampling procedure

5.2.1.1 Broadscale longitudinal survey

A survey was made of macroscopic cyanobacteria and other vegetation at 100 sampling locations distributed throughout the first to fourth order streams of the study site. Observations were made monthly for 12 months (January-December 2011). At each sampling location a 2 m stretch across the full width of the stream was thoroughly examined with the aid of a viewing tube for presence of macroscopic cyanobacteria and other vegetation. The concept of macroalgae as defined by Sheath and Cole (1992) is adopted here. The abundance of each type of macroalga (and other vegetation) was estimated visually in terms of percentage cover using a modified Braun-Blanquet cover scale with the following values: 0 as absent, 1 as <1%, 2 as 1-10%, 3 as 11-25%, 4 as 26-50%, 5 as 51-75% and 6 as 76-100% (Mueller-Dombois and Ellenberg, 1974). Taxonomic composition of each visually distinguishable macroscopic cyanobacterial growth was assessed (Chapter 3). Additional collection of crust material was made to investigate the cyanobacterial component present throughout the system in more detail. Ten 'black' and dark coloured crusts were collected from each stream order and the taxonomic composition of these epilithic communities was investigated.

At each sampling location assessment was made of the dominant substratum type, degree of stream shading and rate of flow. Stream bed substrata were classified using a modified Wentworth scale: bedrock > 4000 mm, boulders> 256-4000 mm, cobbles > 64- 256 mm, pebble >16-64 mm, gravel > 2-16 mm, sand > 0.063-2 mm and silt < 0.063 mm (Harding *et al.*, 2009). The area occupied by each of these size classes was estimated. Degree of shading due to stream banks and riparian vegetation was assigned as one of the following four categories: unshaded, 0-30% shade, partly shaded 30-60% shade and shaded >80% shade. Runs, riffles and pools were defined on the basis of water flow (Harding *et*

al., 2009). Visual comparison of water level, silt deposition and livestock activities were also recorded as indication of past or current disturbances.

5.2.1.2 Small scale mapping

Conspicuous cyanobacterial mats, gelatinous colonies and crusts were monitored at nine locations as indicated in Chapter 2. These locations were selected because each had good accessibility and several types of periphytic cyanobacteria. In the third order stream there were three replicate locations, all of which supported the growth of mats (*P*. cf. *bekesinese* and *O*. cf. *simplicissima*). In Kaituna River (4th order), three replicate locations supported the growth of mats (*P. uncinatum, P. autumnale*), and a further three the growth of gelatinous colonies (*N. verrucosum*, *Nostoc* sp. 1), crust (*Rivularia* spp.) and mats (*P. uncinatum, P. autumnale*). At each location, the macroscopic growths over a selected 21 x 30 cm area of stream bed were mapped using water-proof pens and sheets of mylar transparency paper. A sheet of transparency paper was attached to a transparent perspex viewing box and secured by four screws at each end of the box. The viewing box was held slightly below the water surface to avoid ripples over the area to be mapped. Vertical sides around the box prevented water from flowing over the mylar when pushed below the water surface. Four coloured metal discs were placed on the stream bed at each corner of the area to be mapped. These were securely placed into the substratum to permenantly marked each location. The outlines of visual growths of all macroalgae were mapped as accurately as possible and percentage cover of each type was estimated later using the outlines drawn on the maps. The presence of diatoms was noted when they formed macroscopically visible mats. Mapping was repeated on weekly for 12 months.

5.2.2 Water collection and analysis

Conductivity and temperature were measured *in situ* at every sampling location on every occasion using a field meter, HACH Sension156 multiparameter portable meter. Water samples were collected for nutrient analysis on two occasions (8/07/11 and 11/10/11) from 13 locations (before and after the merger of tributary streams with the study site) as indicated in Chapter 2. Each sample was filtered *in situ* using a sealed sterile syringe and membrane filters (pore size 0.45 µm) into separate 100 mL acid-washed polyethylene bottles. These were kept chilled in an ice chest and transported back to the laboratory.

Concentrations of nitrate-nitrogen (NO_3^--N), ammonium-nitrogen (NH_4^+-N) and dissolved reactive phosphorus (PO_4^{3-}P) were analyzed on an Easy-Chem Plus (Systea Scientific, Anagni, Italy) discrete auto-analyzer. Nitrate was measured via cadmium reduction, ammonium using the phenol-hypochlorite method and dissolved reactive phosphorus using molybdate reduction (APHA, 2008).

5.2.3 Statistical analysis

Relationships between morphospecies composition and environmental variables was assessed by canonical correspondence analysis (CCA) (Rdevelopment, 2011), followed by a Monte Carlo test (499 permutations). Initial correspondence analysis of morphospecies composition gradient length revealed a value > 4. This justified the use of a unimodal response model and CCA (Lepš and Šmilauer, 2003). Variables analysed were as follows: substratum type (bedrock, boulder, cobble, gravel, pebble, sand and silt), shading (shaded, partly shaded and unshaded); flow (run, riffle and pool) and stream order. The position of each of the 100 sampling locations and the date of sampling were also included in the analysis. Conductivity and temperature data were run in a separate analysis with morphospecies composition for data collected for the month of January only (remaining dataset missing).

5.3 Results

5.3.1 Spatial and temporal distribution patterns along the whole study site.

The algal morphological types demonstrated very variable proportions, with some very marked distinctions in their occurrences (Figs. 5.1 - 5.4; Table 5.1). Members of the Nostocales showed distinct spatial restriction to only a small number of locations in the unshaded fourth order stream (Fig. 5.1). *Nostoc* sp. 2, *Cylindrospermum* cf. *muscicola* and *Anabaena* cf. *oscillariodes* were the extreme with presence noted only at one particular location. These three morphospecies also showed seasonal variation with presence noted only in summer to early autumn. A similar pattern of restricted spatial and seasonal distribution was also recorded for *Placoma regulare* occurring in a run of unshaded second stream.

Members of the mat forming Oscillatoriales showed marked spatial and temporal differences in their occurrence (Fig. 5.2). *Oscillatoria* cf. *limosa* appeared to be a summer species with distribution limited to unshaded pools or very slow runs furthest downstream. *Phormidium* cf. *bekesiense* and *O.* cf. *simplicissima* were abundant in third order, partly shaded locations. *Oscillatoria* cf. *simplicissima* was restricted to these locations while *P.* cf. *bekesiense* extended its distribution to three partly shaded locations in the fourth order stream. Both were restricted to pools or very slow runs at all location.

Phormidium uncinatum was widely distributed in a majority of unshaded runs and riffles along the fourth order stream. Cover of *P. uncinatum* mats decreased in winter with pronounced reduction in July. Mats of *P. autumnale* occurring in runs and riffles along the fourth order stream showed spatial and seasonal variation with complete absence in winter to early spring (July-September). Mats of a mixture of *Geitlerinema amphibium* and *Anabaena* cf. *inaequalis* and mats of *P* cf. *irriguum* were restricted to unshaded slow runs and pools respectively in the fourth order stream. Both showed restricted spatial and temporal distribution patterns with the former being a typical summer mat type

while the latter was recorded only in winter. Mats of *P*. cf. *chalybeum* and mats of a mixture of *P*. cf. *subfuscum,* and *P*. *inundatum* were recorded in unshaded runs from the third and fourth order stream respectively. These mats showed restricted spatial and temporal distribution patterns. The preference for fast-flowing water conditions is probably also reflected in the preferred substratum size. *P. uncinatum, P. autumnale, P*. cf. *chalybeum* and mats of a mixture of *P*. cf. *subfuscum,* and *P*. *inundatum* were epilithic on cobbles and pebbles while Oscillatoria cf. *limosa, Phormidium* cf. *bekesiense, O*. cf. *simplicissima,* mats of a mixture of *Geitlerinema amphibium* and *Anabaena* cf. *inaequalis* were epipelic on sand and silt. *Phormidium* cf. *irriguum* was epiphytic on aquatic angiosperm (Chapter 3).

Epilithic black crusts persisted throughout the year scattered in various locations and these increased in distribution to more unshaded locations in the fourth order stream in August to November (Fig. 5.3). Dark purple crust showed a more restricted spatial and temporal distribution pattern, being restricted to only one unshaded second order run with occurrence noted from January to March. Crust of *Rivularia* sp. also showed a restricted spatial and temporal patterns occurring only at six unshaded locations along the fourth order stream with the absence recorded in November and December (Fig. 5.3).

Aquatic angiosperms were more abundant at unshaded locations further downstream with marked reduction in percentage cover observed in mid winter to early spring (July to September) (Fig. 5.3). Extensive cover was prominent in unshaded slow runs and pools with silt as the dominant stream bed substratum. Diatoms persisted throughout the year and were well distributed at all locations. Percentage cover was reduced in May to July with pronounced reduction in percent cover recorded in July at all locations. Maximum cover percentages were prominent in November and December at all locations.

Visible growths of *Vaucheria* and *Batrachospermum* sporophytes were restricted to a small number of unshaded and partly shaded locations in the third order stream (Fig. 5.4). Both were absent in July and their distribution were greatly reduced, each occurring only at one location. Filamentous chlorophytes were well distributed in third and fourth order stream and were prominent in unshaded locations. (Fig. 5.4). Pronounced reduction in percent cover and distribution was recorded in July. *Batrachospermum* gametophyte and moss were prevalent in shaded locations in the first and second order stream. Both showed little change in abundance and distribution throughout the year. Lichen showed restricted spatial and temporal patterns with occurrence noted only at limited locations in the third and fourth order streams and absence in winter.

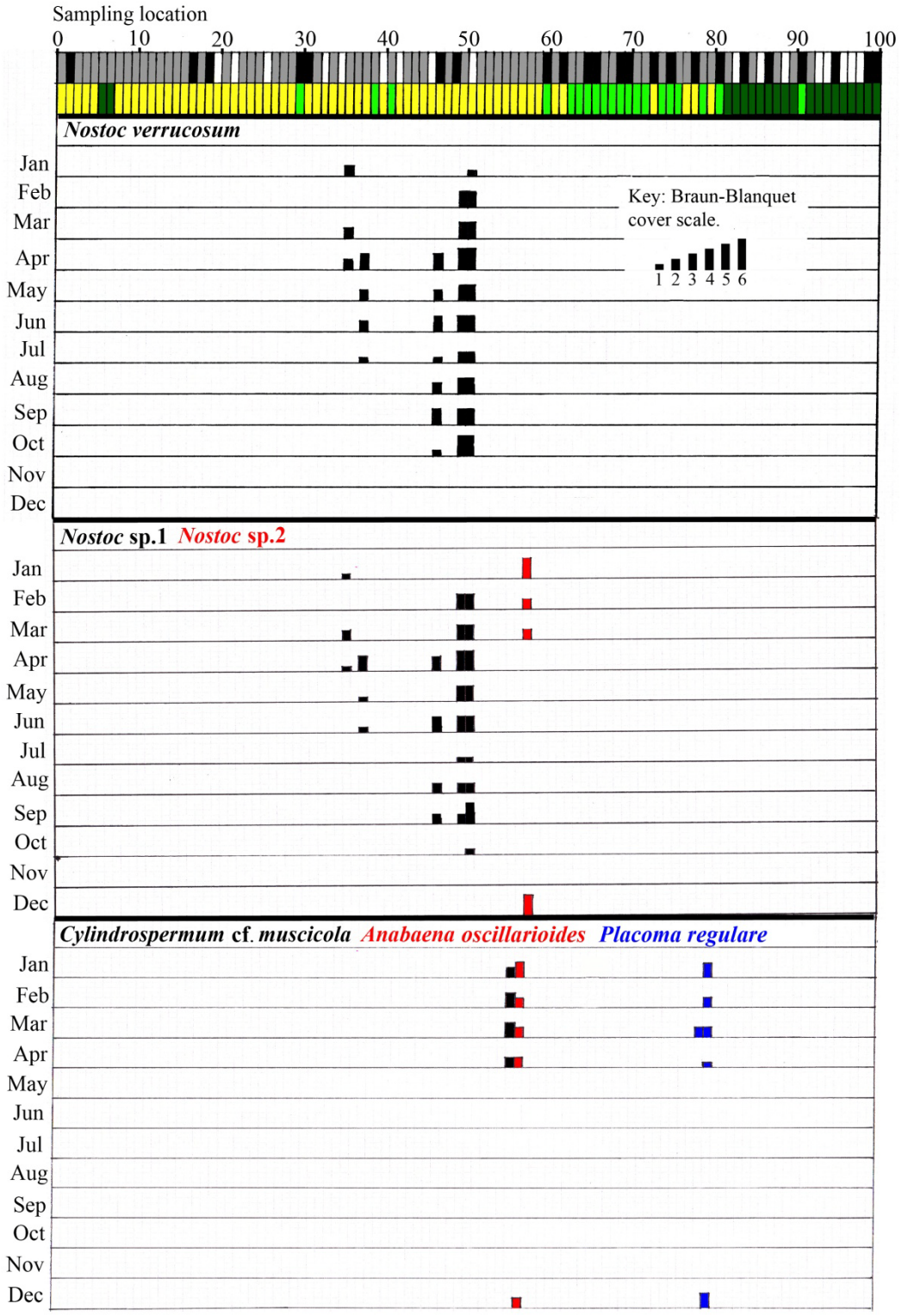

Figure 5.1: Areal cover of mats and gelatinous colonies of cyanobacteria (modified Braun-Blanquet cover scale, see key above) at all sampling locations for January to December 2011. Sampling locations are numbered 1-100 with 1 being the furthest downstream. The row below shows: pool (black), run (grey) and riffle (white). The second row is a subjective estimate of sunlight reaching the stream: unshaded (yellow), partly shaded (green) and shaded (dark green).

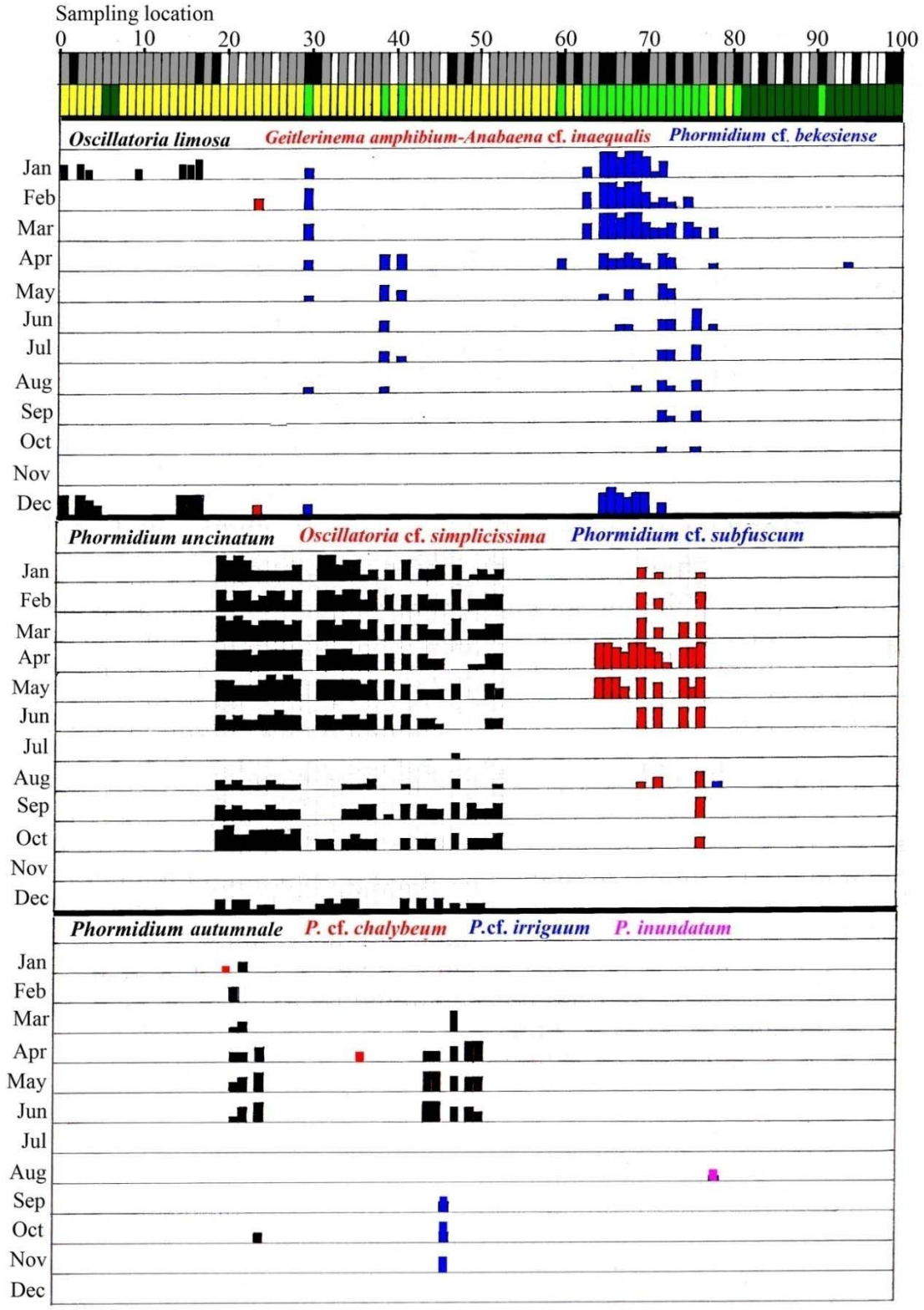

Figure 5.2: Areal cover of mat-forming oscillatorialean cyanobacteria (modified Braun-Blanquet cover scale, see key in Fig. 5.1) at all sampling locations for January to December 2011. Sampling locations are numbered 1-100 with 1 being the furthest downstream. The row below shows: pool (black), run (grey) and riffle (white). The second row is a subjective estimate of sunlight reaching the stream: unshaded (yellow), partly shaded (green) and shaded (dark green).

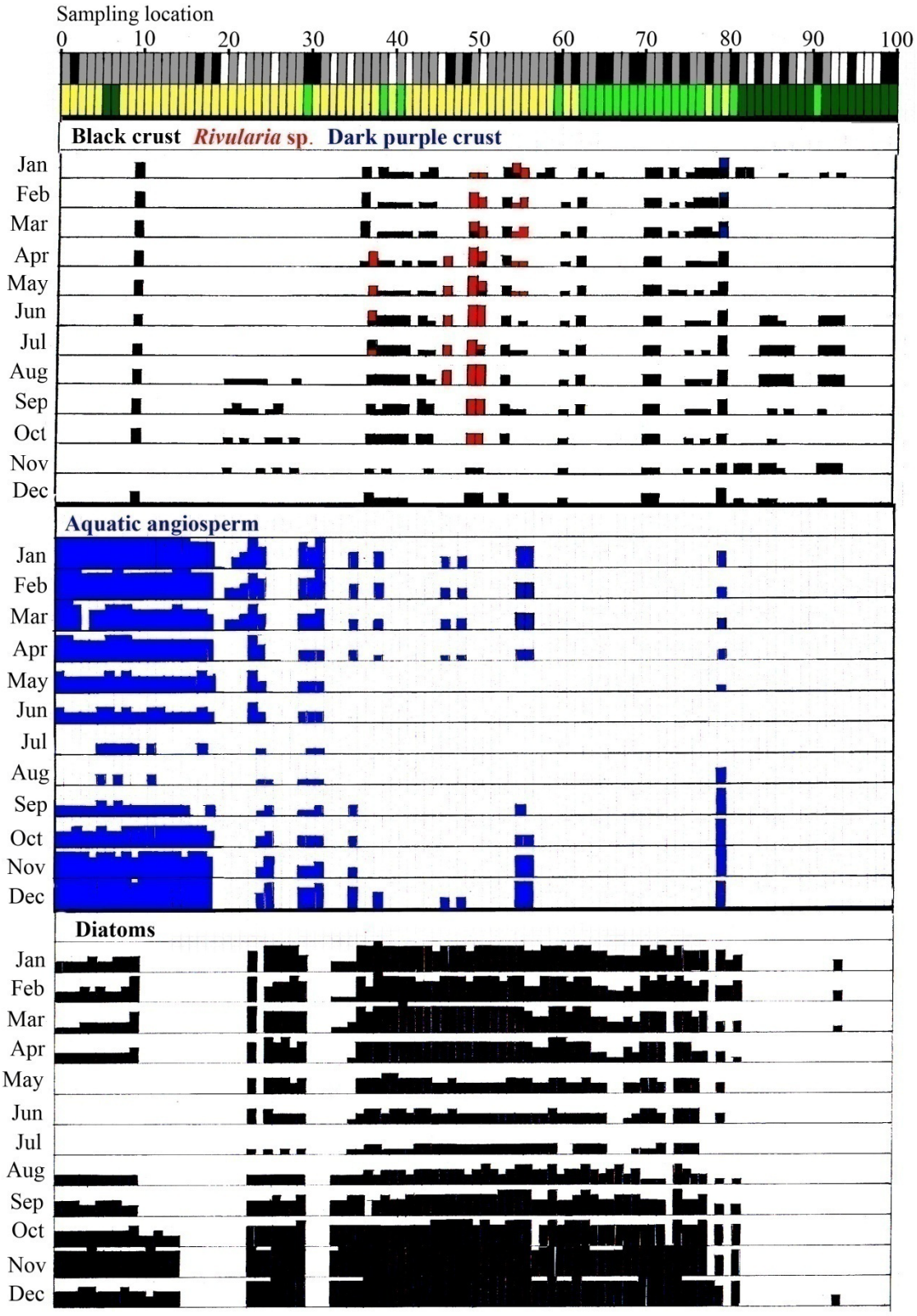

Figure 5.3: Areal cover of crust-forming cyanobacteria, aquatic angiosperms and diatoms (modified Braun-Blanquet cover scale, see key in Fig. 5.1) at all sampling locations for January to December 2011. Sampling locations are numbered 1-100 with 1 being the furthest downstream. The row below shows: pool (black), run (grey) and riffle (white). The second row is a subjective estimate of sunlight reaching the stream: unshaded (yellow), partly shaded (green) and shaded (dark green).

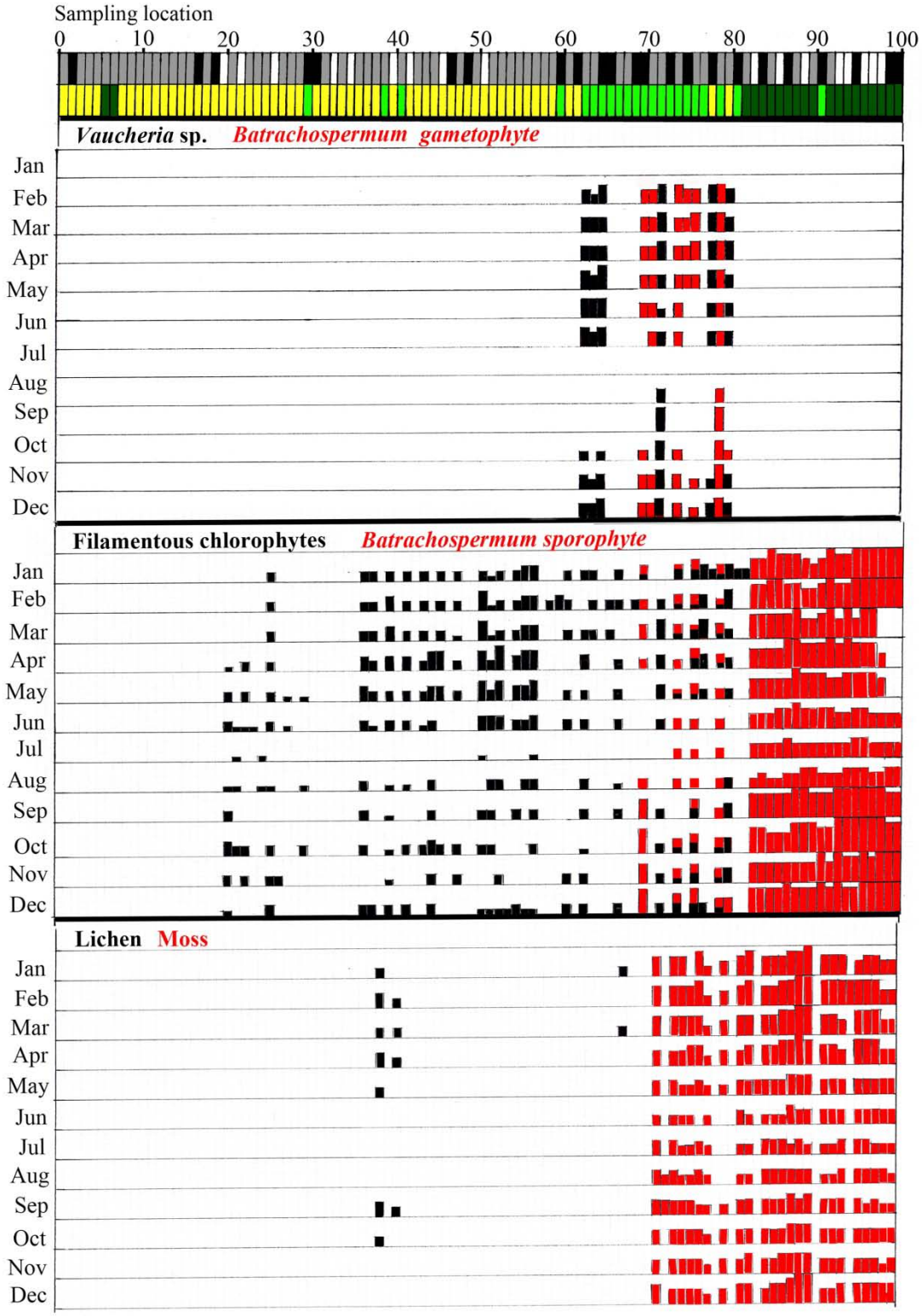

Figure 5.4: Areal cover of different macroalgae, aquatic lichens and moss (modified Braun-Blanquet cover scale, see key in Fig. 5.1) at all sampling locations for January to December 2011. Sampling locations are numbered 1-100 with 1 being the furthest downstream. The row below shows: pool (black), run (grey) and riffle (white). The second row is a subjective estimate of sunlight reaching the stream: unshaded (yellow), partly shaded (green) and shaded (dark green).

Table 5.1: Morphospecies diversity recorded for each of the different stream orders.

Morphospecies	1st order tributary stream	2nd order tributary stream	3rd order tributary stream	4th order Kaituna River
CHROOCOCCALES				
Chamaesiphon amethystinus	+	+		
C. cf. *britannicus*			+	
C. cf. *confervicolus var. confervicolus*	+	+		
C. incrustans		+		
C. subglobosus		+		
Chlorogloea cf. *microcystoides*		+		
Cyanodermatium fluminense		+		
C. cf. *gigas*		+		
Cyanodermatium sp.			+	
Hydrococcus cf. *rivularis*		+		
Placoma regulare		+		
Pleurocapsa cf. *minor*			+	
Pleurocapsa sp.			+	
Radaisia sp.*		+		
Xenococcus sp.*			+	
Xenotholos cf. *kerneri*			+	
OSCILLATORIALES				
Geitlerinema amphibium				+
*G. ionicum**				+
*Heteroleibleinia fontana**	+	+	+	+
H. cf. *kossinskajae**		+		
H. cf. *pusilla**		+		
H. cf. *versicolor**		+		
*Homoeothrix gracilis**				+
*Homoeothrix juliana**				+
H. cf. *varians**		+		
Leptolyngbya cf. *bijugata**				+
O. cf. *simplicissima**			+	
Phormidiochaete sp.*				+
Phormidium autumnale				+
P. cf. *bekesiense**			+	+
P. cf. *chalybeum*				+
P. inundatum			+	
P. cf. *irriguum**				+
P. cf. *subfuscum**			+	
P. uncinatum				+

Table 5.1: continued

Morphospecies	1st order tributary stream	2nd order tributary stream	3rd order tributary stream	4th order Kaituna River
NOSTOCALES				
Anabaena cf. *inaequalis*				+
A. cf. *oscillarioides*				+
Calothrix braunii			+	+
C. cf. *epiphytica**				+
C. parietina			+	+
Cylindrospermum cf. *muscicola**				+
Dichothrix sp.			+	
Nostoc verrucosum				+
Nostoc sp. 1*				+
Nostoc sp.2*				+
Rivularia sp. 1				+
Rivularia sp. 2				+
Trichormus cf. *variabilis*				+

Taxonomic composition of different crust types collected from first to fourth order streams are presented in Table 5.2. Crust communities of the shaded first order stream were dominated by *Batrachospermum* sporophyte with epiphytic *Chamaesiphon* cf. *confervicolus var. confervicolus* being the most abundant cyanobacterium. A similar crust type was also collected from shaded second and third order streams. Crusts in the second order stream have similar epiphytic community as in the first order stream. Epiphytic community in the third order stream were different from that of the first and second order with the presence of *Cyanodermatium* sp. and *Xenococcus* sp. that was not previously recorded (Table 5.2).

Table 5.2: Taxonomic composition of crusts sampled from each stream order (4th order is Kaituna River). Crust types are : 1-cushiony black crust; 2- thin black crust; 3- dark purple hemispherical crust; 4- blackish-brown crust; 5-thick black crust; 6- black crust with distinct circular outline. See Chapter 3 for detailed crust descriptions. Specimens occurring in high abundance are indicated by ++.

	Stream order and crust type												
	First		Second			Third					Fourth		
Morphospecies	1	2	1	2	3	1	2	4	5	6	2	5	6
Chamaesiphon amethystinus	+		+										
C. cf. *britannicus*								+					
C. cf. *confervicolus var. confervicolus*	++		++										
C. subglobosus					+								
Chlorogloea cf. *microcystoides*					+								
Cyanodermatium fluminense				+	+								
C. cf. *gigas*					+								
Cyanodermatium sp.						+							
Hydrococcus cf. *rivularis*					+								

Table 5.2: continued

	Stream order and crust type												
	First		Second			Third					Fourth		
Morphospecies	1	2	1	2	3	1	2	4	5	6	2	5	6
Pleurocapsa cf. *minor*							+				+		
Pleurocapsa sp.							+				+		
Radaisia sp.					+								
Xenococcus sp.						+							
Heteroleibleinia fontana		+		+	++		+				+		
H. cf. *kossinskajae*					+								
H. cf. *pusilla*					++								
H. cf. *versicolor*					+								
Homoeothrix gracilis											+		
H. Juliana											+		
H. cf. *varians*					++								
Phormidiochaete sp.											+		
Calothrix cf. *braunii*									+			+	
C. cf. *parietina*									++			+	
Dichothrix sp.									+				
Rivularia sp. 1										+			+
Rivularia sp. 2										+			+

Thin black crust was the most widely distributed crust-type. The cyanobacterial community of this crust-type differed in taxonomic composition between the different stream orders. *Heteroleibleinia fontana* was the common component throughout the study site. *Pleurocapsa* cf. *minor* and *Pleurocapsa* sp. occurred upstream from *Phormidiochaete* sp., *Homoeothrix gracilis* and *H. juliana* in the fourth order stream and were also present in the third order stream.

Dark purple crusts were noted only in the unshaded second order stream. Cyanobacteria dominated the crust. Species richness was the greatest with 11 morphospecies co-occurring but at different levels of abundance. *Heteroleibleinia fontana*, *H.* cf. *pusilla* and *Homoeothrix* cf. *varians* were the major components followed by the frequently recorded *Cyanodermatium fluminense*. The remaining morphospecies were minor components. Blackish-brown crusts of *Chamaesiphon* cf. *britannicus* were restricted in distribution occurring only at an unshaded location in the third order stream. Heterocyteous crust-forming morphospecies were observed only in unshaded third and fourth order streams.

Different cyanobacterial morphospecies have been collected from different substrata (Table 5.3). The epilithic community is the most diverse with 34 morphospecies compared to five epipelic and eight epiphytic morphospecies. *A. cf. oscillarioides* and *Nostoc* sp. 2 occurred as metaphyton with both

forming soft gelatinous colonies that were neither directly attached to substrata nor freely suspended in the water column but were loosely caught amongst aquatic angiosperms.

Table 5.3: Total diversity across 12 months observed on different types of substrata. Substrata are: Be – Bedrock, Bo – Boulder, Co – Cobble, Pe – Pebble, Gr – Gravel, SS – Sand/Silt and P/MA – Plants/Macroalgae. * indicates metaphytic species loosely associated with aquatic angiosperm.

	Substrata						
Morphospecies	**Be**	**Bo**	**Co**	**Pe**	**Gr**	**SS**	**P/MA**
Chamaesiphon amethystinus							+
C. cf. *britannicus*	+	+					
C. cf. *confervicolus var. confervicolus*							+
C. incrustans							+
C. subglobosus			+				
Chlorogloea cf. *microcystoides*			+				
Cyanodermatium fluminense		+	+				
C. cf. *gigas*			+				
Cyanodermatium sp.							+
Hydrococcus cf. *rivularis*			+				
Placoma regulare	+	+	+				
Pleurocapsa cf. *minor*			+	+			
Pleurocapsa sp.			+	+			
Radaisia sp.			+				
Xenococcus sp.							+
Xenotholos cf. *kerneri*							+
Geitlerinema amphibium						+	
G. ionicum			+	+	+		
Heteroleibleinia fontana	+	+	+	+			
H. cf. *kossinskajae*			+				
H. cf. *pusilla*			+				
H. cf. *versicolor*			+				
Homoeothrix gracilis		+	+	+			
Homoeothrix Juliana			+	+			
H. cf. *varians*			+				
Leptolyngbya cf. *bijugata*			+	+	+		
L. foveolarum			+	+			
Oscillatoria cf. *curviceps*			+	+	+		
O. limosa						+	
O. cf. *simplicissima*						+	
Phormidiochaete sp.			+	+	+		
Phormidium autumnale			+	+			
P. cf. *bekesiense*						+	
P. cf. *chalybeum*			+	+			
P. inundatum		+					
P. cf. *irriguum*							+
P. cf. *subfuscum*		+					
P. uncinatum			+	+	+		
Anabaena cf. *inaequalis*						+	
A. cf. *oscillarioides**							
Calothrix braunii			+	+	+		
C. cf. *epiphytica*							+
C. parietina			+	+	+		

Table 5.3: continued

	Substarta						
Morphospecies	**Be**	**Bo**	**Co**	**Pe**	**Gr**	**SS**	**P/MA**
Cylindrospermum cf. *muscicola*						+	
Dichothrix sp.			+	+			
Nostoc verrucosum		+	+	+			
Nostoc sp. 1		+	+	+			
Nostoc sp.2*							
Rivularia sp. 1		+	+	+	+		
Rivularia sp. 2			+	+	+		
Trichormus cf. *variabilis*						+	
Total	**3**	**10**	**29**	**20**	**9**	**5**	**8**

An overall preference for cobble and pebble rather than bedrock probably also reflects avoidance of shade as bedrock was the predominant substrata in the shaded order 1 and 2 streams. Sand and silt supported only five morphospecies which were recorded from both partly shaded and unshaded locations.

Diversity of macroscopic cyanobacteria was highest in summer (16 types) followed by autumn (14 types) while spring and winter had lowest diversity with only 10 different types (Table 5.4). Total morphospecies diversity in summer and autumn was much higher than in winter and spring due to the occurrence of dark purple crusts in which 11 morphospecies have been identified (Table 5.4).

Table 5.4: Occurrence of the different species of macroscopic cyanobacteria during different seasons. Seasons are: Spring – September to November, Summer – December to February, Autumn – March to May, Winter – June to August.

	Season			
Macroscopic growth	**Spring**	**Summer**	**Autumn**	**Winter**
Gelatinous colonies				
Nostoc verrucosum	+	+	+	+
Nostoc sp. 1	+	+	+	+
Nostoc sp. 2		+	+	
Anabaena cf. *oscillarioides*		+	+	
Placoma regulare		+	+	
Mats				
Oscillatoria limosa		+		
O. cf. *simplicissima*	+	+	+	+
Phormidium autumnale	+	+	+	+
P. cf. *bekesiense*	+	+	+	+
P. cf. *chalybeum*		+	+	
P. inundatum				+
P. cf. *irriguum*	+			
P. cf. *subfuscum*				+
P. uncinatum	+	+	+	+
Geitlerinema amphibium / Anabaena cf. *inaequalis*		+		
Cylindrospermum cf. *muscicola*		+	+	

Table 5.4: continued

	Season			
Macroscopic growth	**Spring**	**Summer**	**Autumn**	**Winter**
Crust				
Black crust	+	+	+	+
Dark purple crust		+	+	
Rivularia crust	+	+	+	+
Total	**10**	**16**	**14**	**10**

5.3.2 Environmental parameters

Water quality data obtained from Environmental Canterbury (ECan) at their monitoring site (at sampling location no. 24 in this study) is presented in Fig. 5.5. Highest conductivity and temperature (20 $ms.m^{-1}$ and 19°C) were recorded in January. Minimum conductivity was in December (12 $ms.m^{-1}$) and minimum temperature in August (5.5°C). Data were missing in February and March following the Christchurch earthquake.

Monthly rainfall recorded at ECan monitoring site was compared with that for TopHouse, upper Kaituna Valley (ca. 250 m). Lowest monthly rainfall for ECan and TopHouse sites were in January with 30.5 mm and 62.0 mm respectively (Fig. 5.5). Highest monthly rainfall (223.5 mm) was in October for TopHouse. Data for ECan monitoring site in October was incomplete as the recorder site was flooded by the river.

Mean monthly flow rate for the main Kaituna River was highest in August (1131.3 $L.s^{-1}$) and lowest in January (92.0 $L.s^{-1}$). Concentration of dissolved reactive phosphorus was highest in May (0.32 $mg.L^{-1}$) and lowest in October (0.011 $mg.L^{-1}$) (Fig. 5.5). Highest concentration of total phosphorus was in March (0.049 $mg.L^{-1}$) while the lowest value was in September (0.022 $mg.L^{-1}$). Concentrations of ammonia, nitrate and nitrite were highest in July with 0.03 $mg.L^{-1}$ and 0.12 $mg.L^{-1}$ respectively while concentrations for both were lowest in January being 0.007 $mg.L^{-1}$ and 0.005 $mg.L^{-1}$ respectively (Fig. 5.5).

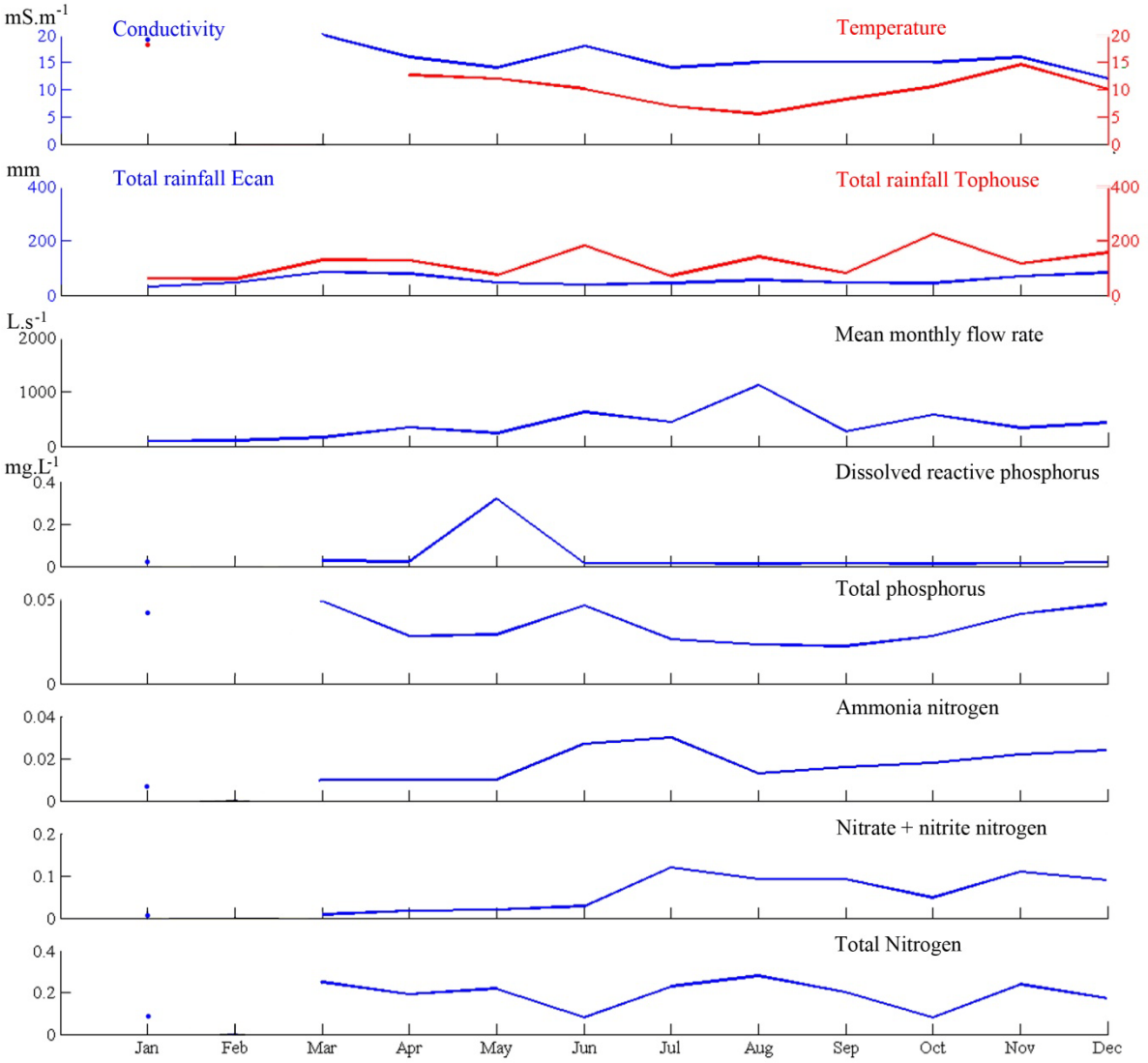

Figure 5.5: Variation of environmental parameters in Kaituna River at the ECan monitoring site from January-December 2011.

Nutrient concentrations were also analysed from 13 sampling locations from the study on two dates (8/07/11 and 11/10/11) (Fig. 5.6). Nutrient concentrations differed depending on the sampling locations. Ammonium concentrations were higher in winter for locations in the first to third order stream while higher values were recorded in spring for locations further downstream. Dissolved reactive phosphorus was high in winter only in the first order stream while the remaining locations showed higher values in spring (Fig. 5.6).

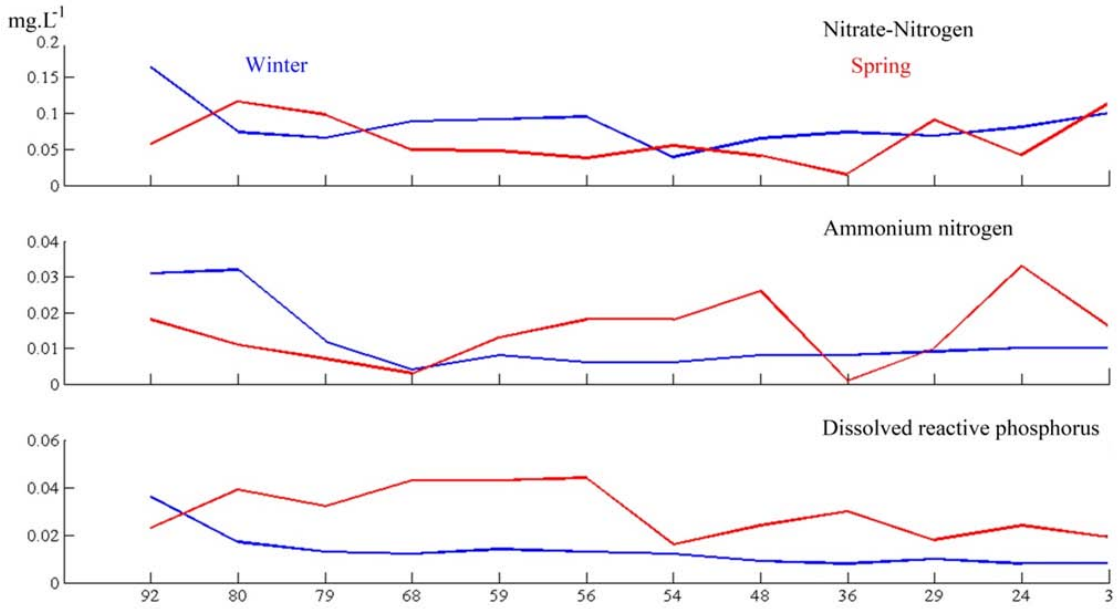

Figure 5.6: Nutrient concentrations at selected sampling locations collected in winter (8/07/11) and spring (11/10/11). Location 92 is in the first order stream, 80 and 79 are in the second order, 68 to 54 are in the third order and 48 to 3 are in the fourth order Kaituna River.

Canonical correspondence analysis (CCA) was used to detect correlations between community structure and the measured environmental variables. The CCA showed that the environmental variables significantly explained 23.16 % of variation in community composition ($P < 0.01$) (Fig. 5.7). The first axis was best described by sampling locations ($F = 37.89$; $P < 0.01$), stream order ($F = 33.09$; $P < 0.01$) and shading ($F = 13.86$; $P < 0.01$). The second axis was best explained by substrata type particularly sand and silt ($F = 13.39$; $P < 0.01$) and boulder ($F = 12.86$; $P < 0.01$). Twelve macroscopic growth comprised of seven mat – types (*Oscillatoria limosa, Geitlerinema amphibium / Anabaena* cf. *inaequalis, Phormidium autumnale, P.* cf. *chalybeum, P.* cf. *irriguum, P. uncinatum* and *Cylindrospermum* cf. *muscicola*), 4 gelatinous colonies (*Nostoc verrucosum, Nostoc* sp. 1, *Nostoc* sp. 2 and *Anabaena cf. oscillarioides*) and one crust (*Rivularia*) had preferences for unshaded locations in the higher stream order. In addition, the gradient contrasted epipelic (e.g. mats of *G. amphibium / A.* cf. *inaequalis* and *O. limosa*) with epilithic growths (Fig. 5.7). Diatoms, filamentous cholorophytes and lichens had similar responses to the environmental variables tested with all being epilithic with preferences for unshaded locations in higher order streams.

Three mat-types (*P. inundatum*, *P.* cf. *subfuscum* and *O.* cf. *simplicissima*), one gelatinous colony (*Placoma regulare*) and one crust (dark purple) preferred partially shaded locations in the lower stream order. Epilithic black crusts were found more often at locations in the lower stream order. *P.* cf. *bekesiense* did not show any clear preferences for any of the measured abiotic factors. Both life-stages of the rhodophyte *Batrachospermum* together with mosses had a strong preference for highly shaded upstream locations (Fig. 5.7).

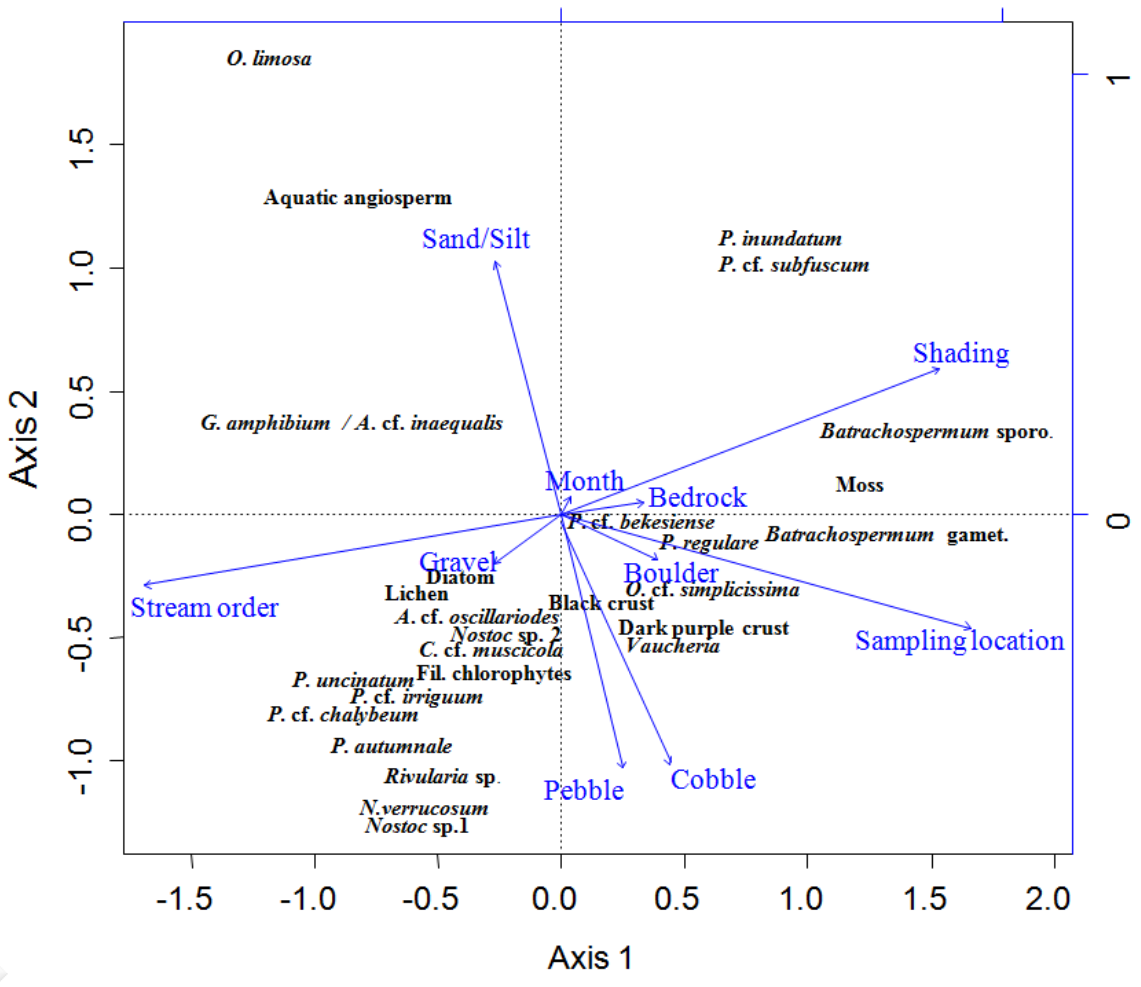

Figure 5.7: Canonical correspondence analysis biplot showing the effects of different environmental variables and time (month) on the occurrence of macroscopic growths of cyanobacteria over a one year period (Jan. - Dec. 2011). The first letter in the name labels indicates the point where each morphospecies lies on the diagram.

A separate CCA was undertaken for water conductivity and temperature data collected in January 2011. Again environmental variables significantly explain variation in community structure ($P < 0.05$) (Fig. 5.8). Stream order ($F = 5.09$; $P < 0.01$) and conductivity ($F = 4.46$; $P < 0.01$) were the most significant variables in explaining the variation in community composition along the first and second axis respectively. Two mat – types (*P.* cf. *bekesiense* and *O.* cf. *simplicissima*), one gelatinous colony (*P. regulare*) and two crust – types (black and dark purple crust) were found at locations in the lower stream order and showed preferences to water with high conductivity. Macroscopic growths with preferences to higher water conductivity were separated to those found in water with lower conductivity values. Higher diversity of macroscopic growth forms especially of the heterocytous cyanobacteria occurred at locations in higher stream orders characterized by lower water conductivity.

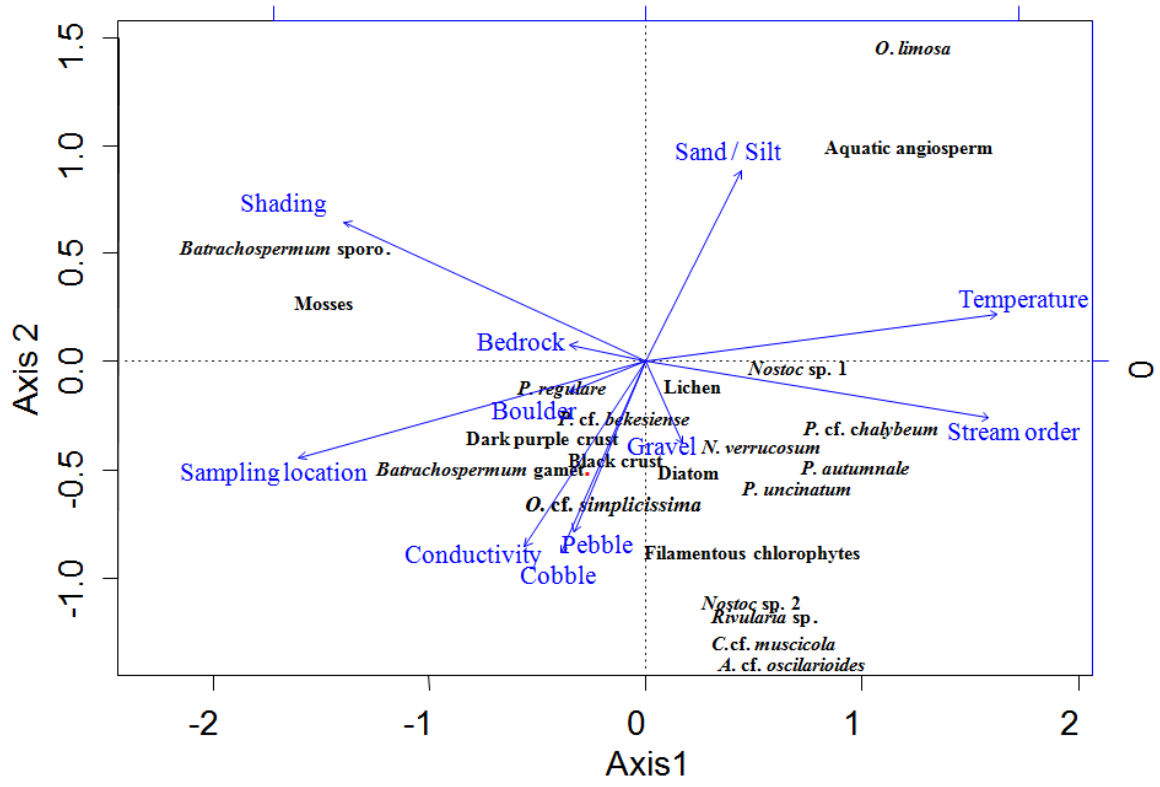

Figure 5.8: Canonical correspondence analysis biplot showing the effects of different environmental variables on the occurrence of macroscopic growths of cyanobacteria during January 2011. The first letter in the name labels indicates the point where each morphospecies lies on the diagram

5.3.3 Small scale spatial and temporal distribution patterns at selected locations

Changes in morphospecies abundance and composition at all nine locations throughout a 12 month sampling period are presented in Tables 5.5 and 5.6. Maps of patterns in their distribution on selected occasions are presented in Figs. 5.8 and 5.9.

Phormidium uncinatum and *P*. cf. *bekesiense* mat types became dominant in fourth and third order streams respectively from mid-January (Table 5.5). Both developed in different microhabitats. *Phormidium uncinatum* mats were epilithic in unshaded runs and riffles while *P*. cf. *bekesiense* mats were epipelic in partly shaded pools and slow flowing runs. Both grew in thickness and extensively covered the stream bed in March with *P*. cf. *bekesiense* extending its cover further in April.

The first appearance of *P. autumnale* and *Oscillatoria* cf. *simplicissima* in the fourth and third order streams respectively differed depending on the location. *Phormidium autumnale* mats were observed at two sampling locations, 23 and 48. Mats were first noted in late March at site 48 while mats at site 24 were first recorded in early May. At site 48, mats of *P. autumnale* predominated throughout the period of low water temperature and high flows while mats of *P. uncinatum* collapsed (Table 5.5, Fig.

5.8). Re-growth of *P. uncinatum* mats at site 48 was noted in early August followed by disappearance of *P. autumnale* mats. At site 23, mats of *P. autumnale* were observed only from May to June before collapsing completely while *P. uncinatum* mats dominated. At location 35, *P. uncinatum* mats were not observed during July (Fig. 5.8).

Mats of *O.* cf. *simplicissima* were observed late January to early February at locations 72, 75 and 76. *O.* cf. *simplicissima* co-occurred with *P.* cf. *bekesiense*, usually as a narrow zone around the periphery of *P.* cf. *bekesiense* mats (Fig. 5.8). At site 75, mats of *P.* cf. *bekesiense* collapsed completely mid-February while mats of *O.* cf. *simplicissima* dominated. Mats of *O.* cf. *simplicissima* collapsed with the drop in water temperature and increase in flow towards the end of June at all locations (Fig. 5.8). Re-growth of *P.* cf. *bekesiense* mats was noted in late July at locations 72 and 76.

Heterocytous morphospecies in Kaituna River (4th order) also varied in the time of their initial appearance at different locations (Table 5.6, Fig. 5.9). *Nostoc verrucosum*, *Nostoc* sp. 1 and *Rivularia* sp. were first observed at location 50 in mid-January. Location 50 was the only location in Kaituna River that supported continuous growth of heterocytous morphospecies up to late October, although colonies of *Nostoc* sp. 1 disappeared by the end of September (Table 5.6). At the remaining two locations, *N. verrucosum*, *Nostoc* sp. 1 and *Rivularia* sp. were observed only in early April (Table 5.6). At location 36, a shift from heterocytous morphospecies to mats of *P. uncinatum* and filamentous chlorophytes was observed towards the end of July (Fig. 5.9). *Nostoc verrucosum* dominated at location 47 up to early October while *Nostoc* sp. 1 and *Rivularia* sp. disappeared in late June. Gelatinous colonies and crusts co-occurred with two different mat types at locations 36 and 50 (Fig. 5.9).

Prior to development of cyanobacterial macroalgae, diatoms were dominant as brownish, easily disturbed mats which covered the river bed. Abundance of diatom mats decreased with increase in cover of cyanobacterial mats but they remained present in the majority of locations (Table 5.5). Diatom mats dominated all locations from November to about mid-January.

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Table 5.5: Percentage cover (Braun-Blanquet cover scale) and species composition of mat-forming cyanobacteria observed weekly during 2011 at selected locations in the 3rd order stream and Kaituna River (4th order). Maps for one week (marked in red) representing each month are presented in Fig. 5.8. No observations were made in weeks indicated by: E – period missed due to earthquakes, S – spate. Percentage cover values are as follows**:** 0 absent, 1 <1%, 2 1-10%, 3 11-25%, 4 26-50%, 5 51-75% and 6 76-100%. Abbreviations *P*. and *O*. represent *Phormidium* and *Oscillatoria* respectively.

Order / Location	Morphospecies	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
			E E	E E E		S		S S	S S		S	S	
4 / 23	*P. uncinatum*	1 2 3 **3**	4 **4**	**6**	**4** 2 3 2	**3** 2 2	2 **2**	**2**	**2** 3	**2** 3 4 4	**4** 2		**2**
	P. autumnale					**2** 2 3	3 3 **2**						
	Fil. chlorophytes					**2** 2	2 3 **2**	**2**		2 2 3			
	Diatoms	5 4 3 **3**	3 **3**		2	2 2	2 2 **2** 2	**2**	**2** 3	**2** 2 3 3	**3** 2 3	3 **2 3**	4 3 3 **4**
4 / 35	*P. uncinatum*	2 3 3 **3**	4 **4**	**5**	5 2 **3** 3	**1** 2 1	**2** 3 3		**2** 2	**2** 3 4 4	**4** 2		**2**
	P. autumnale												
	Fil. chlorophytes				2 **2**								
	Diatoms	4 4 4 **4**	3 **2**		2 2 **2** 2	2	**2**	2 **2**	**3** 3	**2** 2 2 2	**2** 2	4 **2 3**	3 3 4 **5**
4 / 48	*P. uncinatum*	2 3 3 **2**	3 **3**						2 3	**2** 3 4 4	**4** 4		**2**
	P. autumnale			**6**	6 3 3 **3**	4 **2** 3	4 4 **4**	**2**	**2** 2				
	Fil. chlorophytes						2 2 **2**						
	Diatoms	5 5 4 **2**	2 **2**		2 2 2 **2**	2 **2** 2	2 2 **2** 2	**2**	**2** 3	**2** 2 2 2	**2** 2 2	4 **2 3**	4 3 3 **4**
3 / 72	*P.* cf. *bekesiense*	2 3 3 **3**	**4** 3	**5**	**6** 4 4 3	3 2	**2** 3 3	2 **2**	**3** 3	**3** 4 3 3	**2** 2		**2**
	O. cf. *simplicissima*		**2**	**3**	**3** 2 2 2	3 **3** 4	**4** 5 5						
	Diatoms	4 4 3 **4**	**3** 4	**2**		2	**2** 2	2 **2**	**2** 2	**3** 4 4 4	**3** 4 3	2 **3 4**	5 3 3 **4**
3 / 75	*P.* cf. *bekesiense*	2 2 **3**	**4**										
	O. cf. *simplicissima*		**2**	**5**	**6** 2 3 2	3 **3** 3	3 **4** 4						
	Diatoms	5 5 4 **4**	**3** 4	**3**		4 2	2 **2** 2	**3**	**3** 3	**3** 3 4 4	**3** 4 3	2 2 **3**	4 3 3 **4**
3 / 76	*P.* cf. *bekesiense*	1 2 2 **3**	**4** 2	**3**		**2** 3	3 **4** 4	2 **3**	2 **4**	**2** 2 3 3	**2** 2		**2**
	O. cf. *simplicissima*	**2**	**2**	**4**	**4** 2 3 3	4	**2**						
	Diatoms	5 5 5 **4**	**3** 3	**2**		3	2	3 **3**		**4** 4 4 4	**4** 4	3 **2 3**	4 2 3 **4**

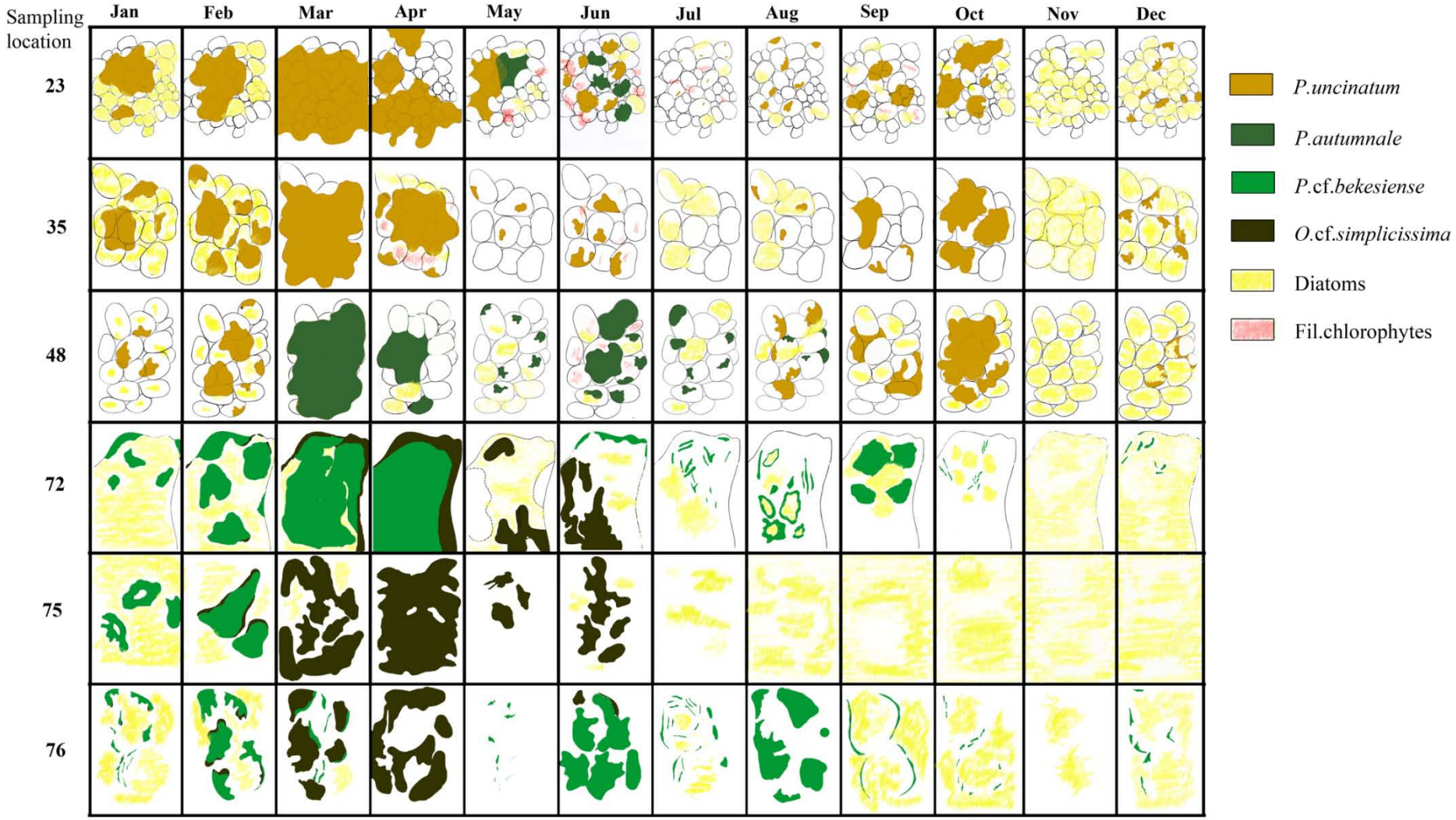

Figure 5.9: Change in abundance and morphospecies composition of mat forming cyanobacteria in Kaituna River (4th order, locations 23, 35, 48) and the 3rd order stream (locations 72, 75, 76). Diatom mats and filamentous chlorophytes are also shown. Each rectangle represents a 21 x 30 cm area of streambed. Abbreviations *P*. and *O*. represent *Phormidium* and *Oscillatoria* respectively

Table 5.6: Percentage cover and species composition of mats and gelatinous colonies of cyanobacteria observed weekly during 2011 at three locations in Kaituna River (4th order). Maps for one week (marked in red) representing each month are presented in Fig. 5.9. No observations were made in weeks indicated by: E – period missed due to earthquakes, S – spate. Percentage cover values are as follows: 0 absent, 1 <1%, 2 1-10%, 3 11-25%, 4 26-50%, 5 51-75% and 6 76-100%. Abbreviations *P*. and *N*. represents *Phormidium* and *Nostoc* respectively.

Location	Morphospecies	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
			E E	E E E		S		S S	S S		S	S	
36	*N. verrucosum*				2 3 **3** 3	3 **3** 3	3 3 3 **3**	**2** 2			*	*	
	Nostoc sp. 1				2 3 **3** 3	3 **3** 3	3 2 2 **2**	**2** 2					
	Rivularia sp.				2 3 **3** 3	3 **3** 3	3 3 2 **2**	**2** 2					
	P. uncinatum	**2** 2	**3** 3	**4**	3 3 **3** 3	3 2	3 3 2	2	**2** 2	**2** 3 3 4	**4**		**2**
	P. autumnale												
	P. cf. *chalybeum*				**2** 3								
	Fil. chlorophytes			**3**	2 **2** 3	2			**2** 2	**2** 3 3 3	**2**		
	Diatoms	5 5 **5** 5	**4** 4	**2**	2 2 **2** 2	2 **2** 3	**3** 3 3	2	**2** 2	**2** 3 3 4	**4** 2 3	2 3 **3**	4 3 2 **4**
47	*N. verrucosum*				**2** 3 3	3 **2** 3	**4** 3 3	**2**	**2** 2	2 2 3 **3**	**2**		
	Nostoc sp. 1				**2** 2 3	2 2	**3** 3 3						
	Rivularia sp.				**2** 2 2	2 1	**3** 2 3						
	P. uncinatum												
	P. autumnale												
	P. cf. *chalybeum*												
	Fil. chlorophytes												
	Diatoms	3 4 **4** 4	**4** 4	**4**	4 **2** 2 2	2	**2**					2 3 **3**	3 2 2 **3**
50	*N. verrucosum*	**2** 3	**3** 3	**4**	4 3 3 **3**	3 **2** 3	**4** 4 4	2 **2**	**3** 3	**3** 3 3 3	**4** 3 3		
	Nostoc sp. 1	**2** 2	**3** 3	**4**	4 3 3 **3**	3 **2** 3	**3** 3 3	2 **2**	**2** 2	**2** 2 2 1			
	Rivularia sp.	**2** 2	**3** 3	**3**	3 3 3 **3**	3 **2** 3	**3** 3 3	2 **2**	**3** 2	**2** 3 3 3	**2** 2 2		
	P. uncinatum	**2** 2	**2** 2	**3**	2 2 2 **2**				**2** 3	**2** 2 3 3	**2**		
	P. autumnale				2 3 **2**	4 2	**3** 3 3	**2**					
	P. cf. *chalybeum*												
	Fil. chlorophytes					2 3	**2** 2 2			**2** 2 3 2	**2**		
	Diatoms	5 4 **4** 3	**3** 3	**3**	4 3 3 **3**	3 **2** 3	**2** 3 3	2 **2**	**2** 2	**3** 3 3 3	**2** 2 2	2 3 **3**	4 3 3 **4**

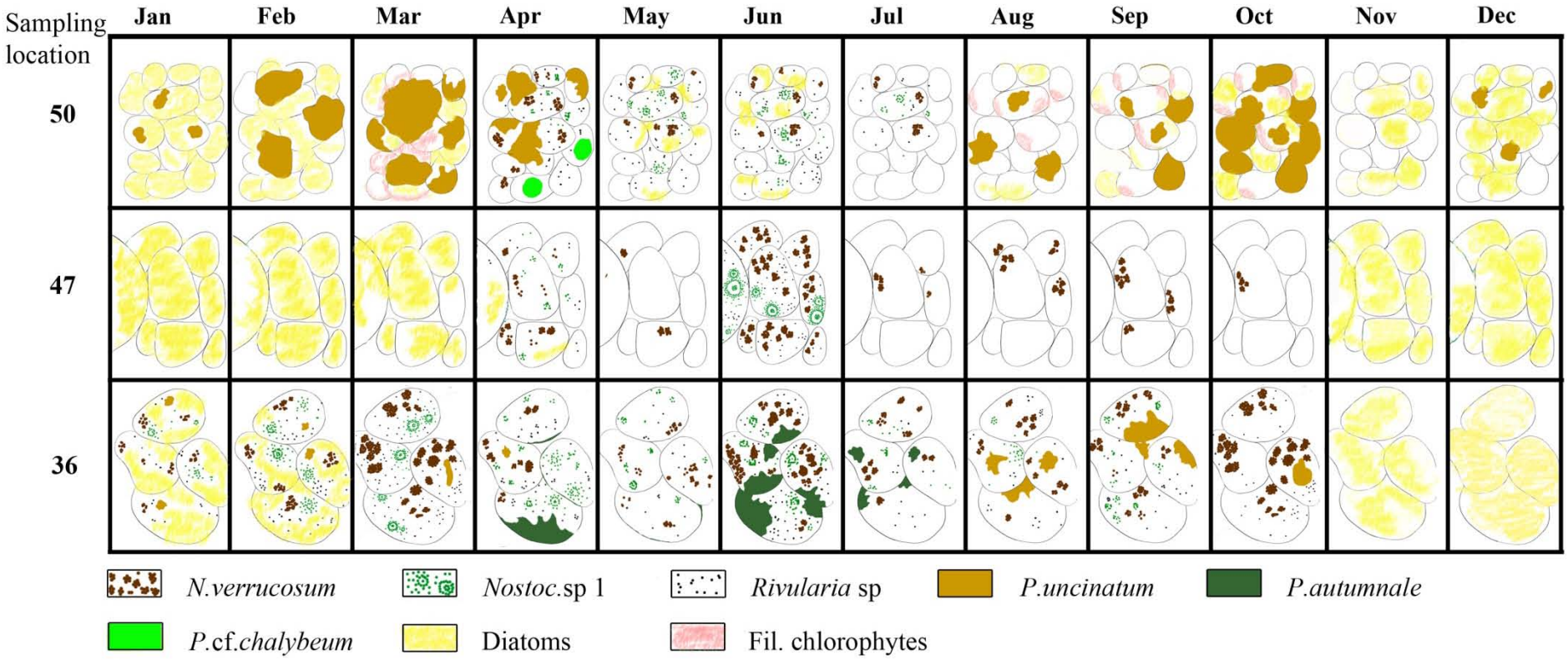

Figure 5.9: Change in abundance and morphospecies composition of mats and gelatinous colonies of cyanobacteria at three locations in Kaituna River (4th order). Diatom mats and filamentous chlorophytes are also shown. Each rectangle represents a 21 x 30 cm area of streambed. Abbreviations *P.* and *N.* represent *Phormidium* and *Nostoc* respectively.

Mean monthly conductivity for all locations is presented in Table 5.7. Conductivity decreased from location 76 to 47 and then increased from 36 to 24. In the third order stream (locations 76 to 72) conductivities were highest in June (144.4-148.5 mg/L) and lowest in August (97.1-97.2 mg/L). In contrast, highest conductivity in Kaituna River (locations 51-24) was in January (88.0-120.3 mg/L) and lowest in September (70.0-77.4 mg/L), apart from location 24 which was lowest in December 72.1mg/L).

Table 5.7: Mean monthly water conductivity at nine locations during 2011. Locations 76 - 72 are in the 3rd order stream, locations 51 - 24 are in 4th order Kaituna River.

Month	Water conductivity at nine locations (mg/L)								
	3/76	3/75	3/72	4/51	4/48	4/47	4/36	4/34	4/24
Jan	145.6	145.1	145.4	88.6	88.0	88.5	91.2	91.3	120.3
Feb	144.2	144.7	144.0	86.8	86.2	86.3	88.0	88.2	120.2
Mar	141.3	141.5	141.7	86.3	86.9	86.0	88.9	88.5	120.6
Apr	116.0	116.0	116.1	82.2	82.5	82.4	85.2	85.5	96.7
May	131.4	144.7	140.7	84.9	85.2	85.0	87.0	87.2	84.4
Jun	147.0	148.5	144.5	84.5	84.8	84.6	86.1	86.0	108.1
July	140.0	142.8	142.2	80.6	80.1	80.2	83.0	83.8	98.0
Aug	97.2	97.2	97.1	78.4	78.8	78.0	81.4	81.9	90.0
Sep	130.0	130.5	130.3	70.0	70.4	70.2	77.4	77.0	90.9
Oct	131.1	130.9	130.6	82.4	82.0	82.1	85.2	85.1	90.6
Nov	138.6	138.0	138.2	83.7	83.9	83.3	87.0	87.6	96.3
Dec	130.7	131.0	130.8	80.1	80.0	80.0	82.8	82.7	72.1

Mean monthly temperature generally increased downstream (Table 5.8). Highest temperatures were recorded in January at all locations (16.0 – 19.0°C). Lowest temperatures were recorded in August with only slight differences between locations (5.3-5.7°C).

Table 5.8: Mean monthly water temperature at nine locations during 2011. Locations 76 - 72 are in the 3rd order stream, locations 51 - 24 are in 4th order Kaituna River.

Month	Water temperature at nine locations (°C)									Monthly range (°C)
	3/76	3/75	3/72	4/51	4/48	4/47	4/36	4/34	4/24	
Jan	16.0	16.2	16.5	17.8	17.9	18.2	18.8	18.9	19.0	16.0-19.0
Feb	14.2	14.5	14.1	15.3	15.8	15.8	16.5	16.3	17.0	14.2-17.0
Mar	12.1	12.0	12.1	13.5	13.6	13.9	14.9	14.7	15.7	12.0-15.0
Apr	11.7	11.6	11.9	12.2	12.5	12.6	12.8	12.3	12.7	11.6-12.7
May	8.9	8.4	8.5	8.8	8.9	8.9	9.0	9.2	12.0	8.4-12.0
Jun	7.1	7.0	7.3	7.8	7.9	7.9	8.1	8.4	10.1	7.0-10.1
July	5.8	5.3	5.5	6.0	6.2	6.3	6.0	6.3	6.9	5.3-6.9
Aug	5.3	5.4	5.4	5.6	5.7	5.8	5.8	5.4	5.5	5.3-5.7
Sep	7.0	7.7	7.1	8.3	8.4	8.4	8.0	8.1	8.2	7.0-8.4
Oct	9.0	9.2	9.1	9.6	9.8	9.9	10.3	10.3	10.5	9.0-10.5
Nov	11.1	11.0	11.1	12.3	12.5	12.6	13.5	13.7	14.6	11.0-14.6
Dec	9.1	9.0	9.3	9.6	9.6	9.7	9.0	9.2	10.0	9.0-10.0

5.4 Discussion

5.4.1 Spatial and temporal distribution patterns along the whole study site

Stream environment provides a great diversity of microhabitats with varying light availability, temperature, stability of substrata, current velocity and physical disturbance that regulates periphyton development (Hill, 1996; Lake, 2000). Distribution patterns observed in cyanobacterial periphyton in this study correlated strongly with different stream orders, shading from riparian vegetation and type of substrata. Downstream increase in stream order coincides with decrease in riparian shading and change in stream bed morphology from bedrocks and boulders to cobbles, pebbles, sand and silt.

5.4.1.1 Light

Light plays a crucial role in determining benthic algal community structure, productivity and taxonomic composition (DeNicola *et al.*, 1992; Hill *et al.*, 2009b; Steinman and McIntire, 1987; Wellnitz and Rinne, 1999). Light reaching the stream bed varies with the distribution and type of riparian vegetation (Hill *et al.*, 1995). Light quality and intensity changes during passage through the riparian vegetation canopy (DeNicola *et al.*, 1992). Since canopy cover differs widely within and among stream systems, light quality and intensity will also vary widely.

Cyanobacterial periphyton in this study had greater cover and diversity in unshaded locations. Although a number of morphospecies were recorded in partly shaded locations, macroscopic growths were more often observed where they received sunflecks. Similar patterns of extended cover in unshaded locations were also observed in diatoms and filamentous chlorophytes. In deeply shaded locations, filamentous rhodophytes and moss predominated. Although macroscopic cyanobacteria were absent at these locations, there were various epiphytic cyanobacteria associated with filamentous rhodophytes. Higher diversity of morphospecies in unshaded locations has been reported by (Quinn *et al.*, 1997a). In their study of streams flowing through pastures in New Zealand, 17 diatom taxa occurred in unshaded channels whilst only 10-12 taxa were present in shaded locations.

In the present study there were differences in the species of cyanobacteria recorded at deeply shaded and unshaded locations. Four morphospecies of coccoid epiphytic cyanobacteria (*Chamaesiphon amethystinus, C.* cf. *confervicolus var. confervicolus, Cyanodermatium* sp. and *Xenococcus* sp.) were the only cyanobacteria in deeply shaded locations. Eight additional morphospecies of coccoid cyanobacteria were found at unshaded locations (*Chamaesiphon* cf. *britannicus, Chamaesiphon subglobosus, Chlorogloea* cf. *microcystoides, Cyanodermatium fluminense, Cyanodermatium* cf. *gigas, Hydrococcus* cf. *rivularis, Pleurocapsa* cf. *minor* and *Pleurocapsa* sp.).

Differences in community composition were also evident in filamentous cyanobacteria. Only two mat-types (*Phormidium* cf. *bekesiense, O.* cf. *simplicissima*) and one crust-type (*Heteroleibleinia fontana*) were recorded from partly shaded locations. At unshaded locations, nine mat-types (*P.*

uncinatum, P. autumnale, P. cf. *chalybeum, P.* cf. *subfuscum / P. inundatum, Oscillatoria* cf. *limosa, P* cf. *irriguum* and *Geitlerinema amphibium / Anabaena* cf. *inaequalis*) and eight crust-types(*H. fontana, H.* cf. *kossinskajae, H.* cf. *pusilla, H.* cf. *versicolor, Homoeothrix gracilis, H. juliana, H.* cf. *varians* and *Phormidiochaete* sp.) have been recorded. Only *H. fontana* occurred in both partly shaded and unshaded locations. All heterocytous morphospecies were only observed in unshaded locations in Kaituna River (4th order).

Changes in community composition at different light intensities have been reported previously. In their study of periphyton in streams of Oregon, Lyford and Gregory (1975) found pennate diatoms to be dominant in shaded locations while filamentous chlorophytes and the diatom *Melosira*, dominated unshaded locations. Similarly, differences have been found in diatom species dominating shaded and unshaded streams in Australia and Nebraska (DeNicola *et al.*, 1992; Roberts *et al.*, 2004). Roberts *et al.* (2004) found no diatom species to occur in both shaded and unshaded locations. *Vaucheria* sp. (Ensminger *et al.*, 2005) and *Stigeoclonium* sp. (Hill, 1996) have been reported to be restricted to unshaded locations. High abundance of filamentous rhodophytes in highly shaded environments (Hill, 1996) is supported by the present study. Some species of *Batrachospermum* are known to decline in abundance at high light intensity (Dillard, 1966).

In contrast, Quinn *et al.* (1997a) found little impact of light on community composition in prairie streams. However, they acknowledged that lack of taxonomic analysis might have contributed to their result. It seems that their records of *Lyngbya* sp. from locations with 0% and 98% shade are unlikely to be the same morphospecies.

Variable light regimes are a common phenomenon in many natural stream habitats (Hill, 1996). Wellnitz and Rinne (Wellnitz and Rinne, 1999) grew periphyton, including *Chamaesiphon* sp., on tiles and exposed these to four different light regimes in a small stream. There was a greater photosynthetic rate in fluctuating light than in constant light, suggesting sunflecks are an important energy source. In the present study, the occurrence of *Phormidium* cf. *bekesiense* and *O.* cf. *simplicissima* mats in sunfleck areas at partly shaded locations may indicate that they preferentially occupy this niche in an otherwise light-limited environment.

Patterns in benthic algal distribution in response to light intensity might therefore reflect possession of specialized traits that enable each species to grow under particular light conditions (Ensminger *et al.*, 2000). Differences in light response by different taxonomic groups have been suggested based on differences in light capturing pigments although most grow best under moderately high irradiances (Hill, 1996). Some cyanobacteria have been reported to grow best at elevate light intensity (Duncan and Blinn, 1989) but others are dominant in shaded streams (Catford *et al.*, 2007). In this study, occurrence of *H. fontana* at both shaded and unshaded locations suggests an ability to grow at a wide range of light intensity. Future studies of the responses of different stream cyanobacteria to different

light intensities could usefully focus on their photosynthetic optima and their ability to photoacclimate. This would increase understanding of why different morphospecies dominate at different light intensities.

5.4.1.2 Types of substrata

Periphyton morphospecies composition on different substrata often differs considerably as some taxa are better adapted to a particular macrohabitat (Round, 1981). The periphytic cyanobacteria in this study showed distinct preferences (Table 5.2). Mats of *O. limosa* and of *G. amphibium* / *A.* cf. *inaequalis* were restricted to sand and silt at locations furthest downstream in Kaituna River (4th order). *Phormidium* cf. *bekesiense* and *O.* cf. *simplicissima* occurred on boulders covered with silt at partly shaded locations in the third order stream. Seven morphospecies were epiphytic on macroalgae and other vegetation whilst the remaining 40 morphospecies were epilithic.

Preferences for different substrata by different periphyton species have been described. A greater diversity of epilithic cyanobacteria was also observed by Potapova and Charles (2005) in their 8 years monitoring study in rivers throughout the United States. Greater diversity was found on wood than tiles in forested streams in Oregon (Sabater *et al.*, 1998). This was considered to be due to the different microhabitats provided by rough and smooth wood surfaces. Other studies have found a higher abundance of algae on rough textured than smooth surfaces of rocks (Downes *et al.*, 2000; Murdock and Dodds, 2007). This preference has been related to better cell adhesion (Murdock and Dodds, 2007; Sekar *et al.*, 2004), more protection from disturbance by grazing and scouring (DeNicola and McIntire, 1991; Elizabeth and Jennifer, 2004) and variations in current regime created as water flows over irregular substrata (DeNicola and McIntire, 1991).

Substrata of different size present different degrees of stability (Power and Stewart, 1987). Stable large boulders and bedrock often support periphyton even where currents are rapid (Uehlinger and Brock, 1991) although fine-grained substrata like mud and sand have also been reported to support high biomass accrual (Iversen *et al.*, 1991; Paul Tett, 1978). Biggs *et al.*, (1999) demonstrated that stream bed stability was of prime importance in determining degree of loss of algal biomass during floods in 12 headwater streams around New Zealand. Stream bed substrata that shift constantly had significant losses of periphyton by abrasion during spates (Biggs and Close, 1989). Algal communities on fine substrata are easily displaced unless they produce mucilage that binds particle together (Madsen *et al.*, 1993).

Bedrock, boulders, cobbles and pebbles were more prevalent in riffles and runs throughout the study site. Sand and silt were dominant substrata in pools and very slow runs. Epipelic cyanobacteria were less diverse than the epilithic community with only five morphospecies recorded. These formed mats that were loosely attached to the substratum, usually where flow was reduced. In Kaituna River,

epipelic mats of *P.* cf. *bekesiense* grew on sand and silt of submerged banks away from the strong flow in the main channel.

Mats of epipelic and epiphytic *O. limosa* occurred only at locations furthest downstream in Kaituna River. Extensive cover of aquatic angiosperms at these locations greatly reduced the opportunity for *O. limosa* to grow over the river bed but, during its peak development in December to late January, mats were common as epiphytes on aquatic angiosperms. According to Komárek and Anagnostidis (2005), *O. limosa* can attached on various substrata or form free floating mats in stagnant or slow flowing waters. Different substrata are also colonized by other Oscillatoriales members (Komárek and Anagnostidis, 2005). *Phormidium aerugineo – caeruleum* (Gomont) Anagnostidis et Komárek 1988 and *Phormidium taylori* (Drouet et Strickland) Anagnostidis 2001 have been recorded as both epipelic and epiphytic from flowing waters. *Phormidium inundatum* recorded from this study was epilithic while records of it being epiphytic also existed from similar environment. *Phormidium corium* (Gomont) 1892 can be found on rock surfaces or as attached algae from streams and rivers. *Phormidium chalybeum* and *P. autumnale* from this study were epilithic but both have been observed growing on sand and silt elsewhere. Similarly, *Phormidium favosum* Gomont ex Gomont 1892 and *Oscillatoria princeps* Vaucher ex Gomont 1892 can both grow on rocks or on sand and silt surfaces in flowing waters.

The epilithic cyanobacteria were more diverse and strongly attached and comprised tightly adherent crusts, gelatinous colonies and thick, cohesive mucilaginous mats. Factors contributing to their higher diversity are discussed further in section 3.4.2.

5.4.1.3 Conductivity and nutrient concentrations

In New Zealand, conductivity has been found to correlate with stream periphyton composition and biomass (Biggs 1990; Biggs and Price, 1987). Different taxonomic groups corresponded to different conductivities in a survey of 100 New Zealand rivers (Biggs, 1990). Communities dominated by the chlorophytes *Ulothrix zonata*, *Stigeoclonium* sp. and *Spirogyra* sp. occurred at low conductivity while those dominated by *Cladophora glomerata* and *Rhizoclonium* were prevalent at high conductivity.

In the present study, conductivity remained high in the first to third order streams and then initially decreased on confluence with fourth order Kaituna River followed by renewed increase downstream. As described earlier, taxonomic composition of the cyanobacterial periphyton differed between these locations. Eleven epilithic crust-forming morphospecies (*Chamaesiphon cf. britannicus, C. subglobosus, Chlorogloea* cf. *microcystoides, Cyanodermatium fluminense, C.* cf. *gigas, Hydrococcus* cf. *rivularis, Radaisia* sp., *Homoeothrix varians, Heteroleibleinia* cf. *kossinskajae, H.* cf. *pusilla* and *H.* cf. *versicolor*) were present at higher conductivity while nine (*Homoeothrix gracilis, H. juliana, Phormidiochaete* sp., *Pleurocapsa* cf. *minor, Pleurocapsa* sp., *Calothrix* cf.

braunii, *C.* cf. *parietina*, *Rivularia* sp. 1 and *Rivularia* sp. 2) were characteristics of low conductivity. In contrast, only two mat-forming morphospecies (*P.* cf. *bekesiense* and *O.* cf. *simplicissima*) occurred at high conductivity.

Changes in nutrient concentrations along the study site and over time appeared to have no effect on macroalgae morphospecies distribution. This has also been reported by Hill and Knight (1988), Mosisch *et al.*(2001) and Hill *et al.*, (2009). In contrast, Perona *et al.*(1998), Douterelo *et al.* (2004), Vis *et al.*, (2008) and Loza *et al.* (2013) showed a significant effect of changes in nutrient concentrations. For instance, oscillatorialean mats dominated locations with high nutrient concentrations (Yu *et al.*, 1995) while low nutrient concentrations favoured growth of heterocytous cyanobacteria (Perona *et al.*, 1998; Douterrelo *et al.*, 2004; Loza *et al.*, 2013). In this study, both mats and gelatinous colonies of heterocytous cyanobacteria co-occurred at three locations (36, 47 and 50) in Kaituna River. These conflicting results suggest that factors other than nutrients are more important in determining distribution of these macroscopic growths.

Hill and Fanta (2009) investigated the combined effect of phosphorus supply and light in the growth and composition of benthic microalgae in experimental streams. Light effects were much stronger than those of phosphorus and resulted in a nearly ten – fold increase in algal biovolume over a range of experimental irradiances compared with a two-fold increase over the range of phosphorus concentrations. They also reported changes in species composition of algal assemblages with increased light intensity. Cashman *et al.*, (2013) conducted an *in situ* experiment to observe the effects of light and nutrient availability on stream periphyton biomass. There was an increase in biomass at greater light intensities while nitrate and phosphate addition showed no significant effect.

Heavily shaded locations have been found to be less favourable for N_2 – fixing cyanobacteria (Elwood *et al.*, 1981). Heterocytous cyanobacteria in this study were absent from the first and second order streams and sparse in the third order stream. Their absence at these locations is attributed to the dense canopy cover. In these shaded locations the energy intensive process of nitrogen-fixation is probably inhibited.

5.4.1.4 Seasonal patterns

There were seasonal changes in morphospecies occurrences in the present study (Figs. 5.1-5.4). *Oscillatoria limosa* and mats of *Geitlerinema amphibium / Anabaena* cf. *inaequalis* were present only in summer (December to February) while *Nostoc* sp. 2, *Cylindrospermum* cf. *muscicola*, *Anabaena* cf. *oscillarioides*, *Placoma regulare* and dark purple crust dominated by cyanobacteria were observed until mid autumn (December to April). The increase in cover during summer of *O. limosa*, *Geitlerinema amphibium / Anabaena* cf. *inaequalis*, *Cylindrospermum* cf. *muscicola*, *Nostoc* sp. 2 and *Anabaena* cf. *oscillarioides* is suggested to be due to reduced current velocity, higher water temperature and greater light intensity.

Mats of *O. limosa, Geitlerinema amphibium / Anabaena* cf. *inaequalis, Nostoc* sp. 2, *Cylindrospermum* cf. *muscicola* and *Anabaena* cf. *oscillarioides* are susceptible to sloughing during increased flows. This is likely due to their growth forms as has been reported by Biggs *et al.* (1998). They observed a change in taxonomic composition and growth form over a gradient in water velocity in four natural and one artificial stream in the South Island of New Zealand. They concluded that with increased velocity, loosely aggregated filaments are the most susceptible to increased skin friction that causes drag and leads to a higher rate of sloughing. Biggs and Thomsen (1995) found similar effects of increased shear stress in experiments on stream periphyton using a laboratory flow tank. They concluded that weakly attached filamentous taxa were more susceptible to minor flow perturbations. This seems to apply to the loosely attached mats of *O. limosa, Geitlerinema amphibium / Anabaena* cf. *inaequalis* and *Cylindrospermum* cf. *muscicola* while the soft colonies of *Anabaena* cf. *oscillarioides* and *Nostoc* sp. 2 growing amongst aquatic angiosperms were equally vulnerable to increased flow.

Although *Placoma regulare* and dark purple crust have growth forms that are likely to be more resistant to high flow rates, they also showed a sharp decline in cover by mid-autumn. Both occurred at the same unshaded second order stream location (80) at the crossing of Pack Horse Hut recreational track. The decline in cover was observed at the same time as increased disturbance of the stream-bed by cattle. An increase in nutrient availability and physical disturbance could be the cause of their decline.

Mats of *O. limosa* and *Geitlerinema amphibium* / *Anabaena* cf. *inaequalis* both were first recorded in December. However, mats of *Geitlerinema amphibium* / *Anabaena* cf. *inaequalis* disappeared in January while *O. limosa* remained abundant and reappeared in February when *O. limosa* was absent. Mats of *Geitlerinema amphibium* / *Anabaena* cf. *inaequalis* have only been recorded from location 24. Other macroscopic growth with similar restricted distribution included mats of *P.* cf. *irriguum* (location 46), *P. inundatum* and *P.* cf. *subfuscum* (location 79), *Cylindrospermum* cf. *muscicola* (location 56), *Anabaena* cf. *oscillarioides* (location 57), *Nostoc* sp. 2 (location 58), dark purple crust (location 80) and *Placoma regulare* (location 80 with extended distribution to location 79 only in March). These macroalage also showed distinct seasonal variation. *Phormidium* cf. *irriguum* occurred only in spring (September to November). Prior to its appearance, diatoms dominated at its single location. With regular occurrence of spates in winter, filamentous diatoms were washed away allowing colonization of available substrata by *P.* cf. *irriguum* in spring. *Placoma regulare, Anabaena* cf. *oscillarioides, Nostoc* sp. 2, *Cylindrospermum* cf. *muscicola* and dark purple crust also showed seasonal variation occurring from January to March and December (summer to mid autumn) while *P. inundatum* and *P.cf. subfuscum* were only observed in August (late winter).

The reasons behind these patterns would require further investigation. Were these morphospecies governed by specific abiotic factors that were only present at the selected location? Could differences in grazing activity at different locations and seasons play an important role in determining the distribution pattern? Microscale studies of changes in environmental factors within each sampling location and effects of grazing would be of interest in understanding these distribution patterns.

The remaining cyanobacteria in this study were present throughout the year but with distinct reductions in cover following spates (Fig. 5.9). A similar pattern was evident for filamentous chlorophytes, diatoms and aquatic angiosperms. Spates were more intense in spring and winter but following these, cyanobacteria re-appeared within three to four weeks. In summer and autumn, less intense spates also caused washout of macroscopic growths of cyanobacteria. These then recovered more rapidly than in spring and winter. This suggests spates were the main source of temporal variation as has been observed previously in New Zealand streams and rivers (Biggs, 1990; Biggs and Gerbeaux, 1993; Biggs and Smith, 2002; Biggs and Thomsen, 1995).

Spates have a major impact on periphyton as they scour and reset biomass to very low values (Mosisch and Bunn, 1997). In this study, spates during July to October caused loss of periphyton at all locations (Figs. 5.1-5.4). Smaller increases in flow caused only partial removal of periphyton with rapid re-growth noted after a week of a flood peak (Fig. 5.10 July and August - regrowth of mats the following week after spates). Similar patterns of re-growth were observed by Biggs and Smith (2002) in 12 gravel bed streams around New Zealand. They found periphyton biomass to be lower after a spate but that did not always reduce morphospecies richness. They recorded 26 periphyton morphospecies from Victoria Stream a day after a flood event compared with similar species diversity at the same sites before the flood. This was attributed to community resistance to disturbance with diatoms and some filamentous green algae being firmly adherrent to their substratum.

Occasional low flow periods of several weeks duration in winter resulted in extensive accumulation of periphyton suggesting that variation in flow is more prominent than seasonality in this study. A major spate following a storm in late October resulted in all macroscopic cyanobacteria being removed (Fig. 5.8 and 5.9). This was followed by re-colonization by diatoms in early November (Fig. 5.8 and 5.9). Effects of major floods on periphyton seem clear but effects of smaller floods can be modulated by substrata stability with communities on sand and silt being most affected (Elósegui and Pozo, 1998). Detailed discussion of the relationship between spates and periphyton community on the different substrata is presented in section 3.4.2.

Cyanobacterial morphospecies richness was higher in summer and autumn than in spring and winter (Table 5.3). A decline in morphospecies richness was observed in late spring (November) when

periphyton at most locations was dominated by diatoms. This late spring decline was after the major flood event in October. Suren *et al.,* (2003) reported a decline in taxonomic richness during summer in Waipara River, Canterbury with periphyton changing from diatoms and cyanobacteria to one dominated by filamentous chlorophytes. They attributed this to interspecific competition for resources (nutrients) during summer low flows. In this study, the dominance of diatoms following the flood event reflects the findings of Biggs and Smith (2002). They found rapid (days to a week) accrual of periphyton diatoms following spates. This was dominated by taxa with high rates of immigration and reproduction and by taxa resistant to disturbance, probably derived from local refugia. In the present study, diatoms appeared to be more resilient than cyanobacteria and chlorophytes. Diatoms have been previously reported to colonize bare substrata more quickly than other algae and tend to dominate young assemblages (Hill and Fanta, 2009).

5.4.2 Small scale spatial and temporal distribution patterns.

Small scale observations were made at nine locations of the preferences of different morphospecies for different substrata and their recolonization ability after spates. As discussed in 3.4.1, strong preferences for particular substrata were apparent. *Phormidium* cf. *bekesiense* and *O.* cf. *simplicissima* mats were affected by silt deposition. Although *P.* cf. *bekesiense* mats occurred throughout the year at locations 72 and 76, the sharp decrease in cover at location 75 in July is attributed to decreasing silt deposition on boulder surfaces (Fig. 5.8). Conversely, colonies of *N. verrucosum* and *Nostoc* sp. 1 declined in May, July August and October with increase of fine sediment on boulders in a pool at location 47 (Fig. 5.9). Stancheva *et al.,* (2012) reported similar decline in *N. verrucosum* with an increase in fine substrata in streams of southern California. Silt deposition reduces the area available for colonisation on more stable substrata (Shortreed and Stockner, 1983).

In this study, periphyton accumulation was greater on horizontal than on vertical surfaces. This is consistent with the observations of Murdock and Dodds (2007) on various types of substrata with different degrees of roughness deployed in a prairie stream. Knott *et al.,* (2004) observed preferences for particular orientations of substrata in different morphospecies. They found epibiota including algae from subtidal reefs were usually very different on vertical and horizontal surfaces but also noted variation in these assemblages within a particular orientation and between reefs. They highlighted that such variations caused difficulty in detecting patterns especially when assemblages on horizontal surfaces were the same as those colonizing vertical surfaces. The complexity of preferences for different orientations of substrata by different organisms, including cyanobacteria, is worth further investigation.

In this study, more cyanobacterial morphospecies occurred on cobbles and pebbles than on sand and silt. Different morphospecies coexisted on a single substratum at most locations. High

morphospecies diversity is best illustrated at location 36 (Fig. 3.8) and 50 (Fig. 5.9) in April. At both locations, during a period of steady flow, five morphospecies comprised of gelatinous colonies, crust and tightly woven mats co-occurred on a single cobble.

The diversity of the different macroscopic growth forms observed in this study is predicted to be related to the texture of the substrata. Sub-millimetre scale surface irregularities, such as pits, crevices and protrusions create heterogeneous microhabitats across a single substratum which may result in colonisation by diverse species of algae (Murdock and Dodds, 2007). Bergey (2005) found small epilithic diatoms to obtain refuge from disturbances in pits but these may deprive them of sufficient light and nutrients. Downes *et al.,* (1998) found stones with rough surfaces and few large crevices supported high abundance of the filamentous rhodophyte *Audouniella hermannii*. The small number of large crevices reduced gazing activity that would otherwise support high abundance of gazers (Downes *et al.*, 1998). Rougher surfaces also have deeper and steeper slope pits creating a mosaic of light intensity across a small area that could possibly allow morphospecies with different light requirements to coexist (Downes *et al.*, 1998).

In this study, macroscopic growths influence availability of the surface of their substratum for development of other cyanobacteria. For instance, cyanobacteria at location 47 (Fig. 5.9) developed in mid-autumn (April) compared to the same morphospecies appearing in midsummer (January) at locations 50 and 36. The prolonged dominance of diatoms at location 47 could be the reason for this difference. At locations 50 and 35, diatom cover decreased gradually from November to January with the increase of cyanobacteria. However, at location 47, diatom cover was prominent from November to March. The filamentous diatoms appeared to maintain their position and extensive cover as over-storey in the stagnant pool. Their growth probably reduced light and nutrient availability at the substratum surface and that could have prevented cyanobacterial growth. Cyanobacteria were observed in the second week of April following two days of rain that caused a spate and the disappearance of the diatoms.

Changes in cover of cyanobacteria following spates have been noted (Tables 5.2 and 5.3). As discussed previously, these changes do not necessarily result in total loss of periphyton but often cause a large reduction in cover (Figs. 5.8 and 5.9 for the months of May, July and August). Cyanobacterial mats were more affected by spates than gelatinous colonies and crusts. Re-growth of mats occurred from remaining small patches at each location. This was typical of mats on cobbles where regrowth started from remnant patches of mat that were attached to small accumulations of sand and silt resting on cobble surfaces. More extensive epipelic mats appeared to be completely washed away during spates, presumably due to their weak attachment. However, patchy mats found at these same locations in as little as 5 days after a spate suggests that they could have been buried under silt brought down in the flood. This suggests that the gliding motility of these morphospecies

could have repositioned buried trichomes to the silt surface. According to Stal (1995), motility in mat-forming cyanobacteria is of great importance in enabling them to position themselves in optimal light following self-shading that occurs in dense aggregations of trichomes. The epipelic mat morphospecies (*P*. cf. *bekesiense* and *O*. cf. *simplicissima*), both of which have rapid gliding motility could respond similarly to regain optimal light intensities.

Patterns observed at this small scale warrant further investigations. Experiments could test the ability of mats to survive under silt and then move back to the surface. From being under what depth of silt are different morphospecies able to return to the surface. The reasons for the growth of *O. cf. simplicissima* mats as a narrow zone around the periphery of *P. cf. bekesiense* mats are unknown. Perhaps there is a competitive interaction taking place. Laboratory experiments using isolates of both species growing together at different light intensities and nutrient concentrations might provide some clues.

Chapter 6

Concluding discussion

6.1 Diversity of periphytic cyanobacteria

The first aim of this study was to investigate the taxonomic diversity of all macroscopic periphytic cyanobacteria in the study site. Fifty-six morphospecies have been identified. Twenty-nine of these are new records for New Zealand (Chapter 3). Epilithic crusts were the most diverse with 23 morphospecies compared to 16 from mats, 5 from gelatinous colonies and 7 epiphytes. The high number of new records from this study reflects gaps in knowledge of periphytic cyanobacteria despite the large number of excellent detailed studies on periphyton in streams and rivers around New Zealand. The high number of genera from epilithic crusts that are rarely recorded worldwide and are poorly known indicates a need for detailed studies of this community globally.

Identifications of morphospecies were made using regional floras (Geitler, 1932; Desikachary, 1959; Komárek and Anagnostidis, 1989, 1998, 2005;McGregor, 2007; Whitton,2011). Whenever possible the taxonomic system and morphospecies concepts of Komárek and Anagnostidis, (1989, 1998, 2005) were used. Since floristic accounts are only available for a restricted number of geographical regions, and mostly for Europe, studies made outside those regions (Sheath and Cole, 1992; Skinner and Entwisle, 2001; Saha *et al.*, 2007; Caisová *et al.*, 2009) have relied heavily on those floras. This has probably resulted in identifications to morphospecies being made even if differences between specimens and the descriptions in the floras should have indicated some doubt. In this study, a more conservative approach has been followed. Nine cyanobacteria could be identified only to the generic level as no morphospecies in the literature conformed closely enough to the specimens. For 21 cyanobacteria there were relatively slight differences in morphology from descriptions of the closest morphospecies in the literature. In these cases, the term "cf." has been applied in front of the species name in order to indicate doubt that it is a true member of that species. These 30 cyanobacteria could either be new, genetically distinct species or environmentally induced morphological variants of the most similar morphospecies in the literature. To confirm this would require in depth studies of each involving specimens and isolates from other regions and both detailed morphological studies and molecular phylogenetics. This was beyond the scope of the present study. Two strains assigned to *Nostoc* as unidentified species *Nostoc* sp. 1 and *Nostoc* sp. 2 were subjected to 16S rDNA analysis (Chapter 4). *Nostoc* sp. 1 formed a cluster with strains of *Nostoc* that are lichen phycobionts while *Nostoc* sp. 2 was not related to any of the *Nostoc* clade in the phylogeny despite its morphological

resemblance to *Nostoc* (Chapter 4). Further investigation needs to be carried out to determine the possibility of it being a new genus.

Both morphological and molecular phylogenetic approaches were applied to the 12 strains that were isolated into culture. It was evident that there were inconsistencies between the traditional concept of families and clustering of strains within molecular phylogenies (Chapter 4). The morphological approach places *Placoma regulare* and *Chamaesiphon subglobosus* into two distinct families (Hydrococcaceae and Chamaesiphonaceae). However, 16S rDNA molecular phylogeny revealed that *P. regulare* was a close relative of *Chamaesiphon subglobosus* PCC 7430 (Chapter 4). Conversely, traditional taxonomy based on morphology assigns *Heteroleibleinia fontana* and *Tapinothrix* sp. to the same family, Heteroleibleiniodea, and separates the two genera based only on elongation of the apical cell. However, a molecular phylogeny based on 16S rDNA showed them to be distant relatives.

Six oscillatorialean morphospecies in this study (*Phormidium autumnale*, *P.* cf. *bekesiense, P. inundatum, P.* cf. *subfuscum,* two strains of *P. uncinatum* and *Oscillatoria curviceps*) had distinctly different ITS sequence compositions. A comparison of the morphology of field specimens and cultures showed no change in apical cell morphology for any of these morphospecies. Also, in four there was little difference in the trichome widths. However, *P.*cf. *bekesiense* and *P*. cf. *subfuscum* did differ in trichome width between field specimens and cultures (Chapter 3). Morphological comparison between strains used in this study with strains of similar ITS composition from other studies showed highly variable features (Chapter 4). Cyanobacteria often show changes in their morphology when maintained in cultures (Whitton, 2011). This could be due to genetic selection of mutations that are most successful in the culture environment. Therefore, identifications could be problematical if based solely on strains from cultures and for which there are no records of the morphology of field specimens.

Comparison of the algal flora from this study with others conducted in streams worldwide showed only six morphospecies at the maximum to be common to this and any one of those other studies (Table 3.2). The differences observed in species diversity and the frequent occurrence of rare taxa may be associated with the geographic isolation of New Zealand. Species common elsewhere might have difficulties in dispersing to New Zealand. Species that have dispersed to New Zealand could diverge in morphology from their close relatives overseas. Alternatively, the studies that have been used in the comparison could have been made where environments differ substantially from Kaituna Valley, e.g. different geology and soils, and could have selected for different assemblages of species. Heavy and irregular rainfall, steep upper catchments with low biomass native forest and lack of deciduous trees have been suggested to be factors differentiating New Zealand stream ecosystems from those elsewhere (Winterbourn *et al.*, 1981).

6.2 Temporal and spatial patterns in distribution

The main finding was that there were distinct patterns in periphytic cyanobacterial distribution in space and time at the study site (Chapter 5). Environmental conditions selected for distinct assemblages occurring at different locations with microhabitat characteristics accounting for variability in the distribution patterns (Chapter 5). Light intensity, types of substrata and conductivity were the most significant factors influencing morphospecies composition of assemblages at each location. Distinctly different morphospecies inhabited shaded and unshaded habitats, rocks and sediments, and waters of high and low conductivity (Chapter 5). The results support the notion that everything is potentially everywhere but the environment selects which morphospecies occur at a particular location (Finlay *et al.*, 2002).

It was expected that some morphospecies would have broad environmental tolerances and would be found throughout the study site. *Heteroleibleinia fontana* was the only morphospecies that displayed this pattern and it occupied a variety of microhabitats (Chapter 5). The majority of cyanobacteria were restricted to a limited range of microhabitats. If the environmental factors causing these different patterns were understood then these morphospecies would have potential as biological indicators.

6.3 Future work

The confident identification of many of the cyanobacteria awaits further study of their morphological, ecological, and molecular phylogenetic characteristics. Only 12 morphospecies from this study have been subjected to a polyphasic assessment. The remaining morphospecies were not isolated into culture and many are rare and poorly known taxa. The requirements for growth in culture of many cyanobacteria are still unknown. Further investigations to ascertain these requirements would allow polyphasic studies to be achieved.

In order to investigate how typical the Kaituna flora is of other catchments, studies of similar intensity and focus are needed in other catchments on Banks Peninsula and elsewhere in New Zealand. Only then will the full diversity of cyanobacteria in flowing waters be revealedand their relationship to environmental factors be clearly understood. Banks Peninsula has more than 30 other catchments of comparable size and with similar volcanic geology and soil types, but with different vegetation cover and intensities and types of human activities. This makes the region excellent for the investigation of the effects of catchment vegetation and human activity on diversity and distribution of periphytic cyanobacteria. The extension of such a study to regions of different geology and land use activity would be valuable. It is likely that there would be discoveries of further morphospecies new to the New Zealand flora.

Mats of Oscillatoriales are widespread in streams and rivers around New Zealand. Whether mats with different macroscopic appearance that correspond to different microscopic components, as observed in this study, also exist in other systems has barely been investigated. As oscillatorialean mats are well-known to potentially produce toxins, it would be worth investigating mats in the Kaituna catchment for toxin production. This might differ amongst mat types but also within a particular mat type over time.

Nostoc is a globally widespread genus frequently encountered as macroscopic colonies in a diverse range of habitats. Their diversity in New Zealand has not yet been subjected to a polyphasic study. Colony morphology and microscopic characteristics of field specimens and cultured specimens, combined with molecular phylogenetics would help reveal their true diversity. *Nostoc* sp. 1 from Kaituna River could possibly be derived from a phycobiont of local lichens (Chapter 4). If this is so, then genotypes of stream populations might be influenced by the distribution of lichen species. Sampling and mapping of these is recommended for future work.

On a global scale, the ability to assess the geographical distribution of cyanobacterial morphospecies is restricted by taxonomic uncertainties (Chapter 3). It would be worthwhile to compile information from the literature on diversity of cyanobacteria from streams and rivers worldwide and to assess the identifications made and the nomenclature and taxonomic systems used. Such an exercise might enable recognition of cosmopolitan and endemic morphospecies and reveal gaps in taxonomic understanding as well as regions of under-sampling.

In this study, observations made on small, defined areas of the stream bed revealed a strong impact on the diversity of cyanobacteria of substratum type, size and surface texture (Chapter 5). This method could be further developed and improved by making detailed measurements of microenvironmental factors such as current flow, turbulence and light intensity. Differences in these between microsites are likely to result in the occurrence of different macroscopic growths. Such investigations would provide information on the requirements for colonization by different morphospecies. Only then would there be better understanding of the patchiness of morphospecies distribution between different substrata.

This study has provided a baseline for further investigation of long term change in composition of the cyanobacterial flora and its temporal and spatial distribution in the study site. Two possible changes that could affect cyanobacteria are those of local human activities and of regional climate. Two studies have examined long term change in the total periphyton flora, one in a stream on Vancouver Island (Shortreed and Stockner, 1983) and one in a Norwegian river (Lindstrøm *et al.*, 2004). The first of these used artificial substrata that probably selected for species most able to grow on the plastic substratum. The second examined periphyton on natural substrata and found weak but significant trends that were linked to a warming climate and increasing nutrient concentrations.

Application of techniques similar to those used in the present study could usefully be applied to streams and rivers elsewhere. The problems of using artificial substrata would be avoided and the potential for use of readily distinguishable macroscopic growths of cyanobacteria as environmental indicators would be strengthened.

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